PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/47, C12Q 1/68, G01N 33/50

A2

(11) International Publication Number: WO 98/07850

(43) International Publication Date: 26 February 1998 (26.02.98)

(21) International Application Number: PCT/EP97/04599

(22) International Filing Date: 22 August 1997 (22.08.97)

(30) Priority Data:
2,183,901 22 August 1996 (22.08.96) CA

(71)(72) Applicants and Inventors: BERGMANN, Johanna, E. [DE/DE]; Mörikestrasse 22, D-22587 Hamburg (DE). PREDDIE, Enrique, R. [CA/CA]; 4875 Dufferin Street, Montreal, Quebec H3X 2Z2 (CA).

(81) Designated States: AU, CH, CN, JP, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: AGENTS FOR PRE-SYMPTOMATIC DETECTION AND THERAPEUTIC TARGETING OF ALZHEIMER'S DISEASE AND DOWN SYNDROME IN HUMANS

(57) Abstract

Agents and methods for the diagnosis and therapy of Alzheimer's disease and the related condition Down syndrome are disclosed. Such agents include four genes located within the region of human chromosome 21 occupied by the APP gene, which are exclusively expressed in Alzheimer's disease or Down syndrome, respectively, the proteins encoded and expressed by these genes, the nucleic acid molecules influencing their expression, and endogenous antibodies produced in humans with Alzheimer's disease and Down syndrome against the above proteins. Also disclosed are antibodies and nucleotides to detect the presence of the proteins and nucleic acids in humans.

TITLE OF THE INVENTION:

AGENTS FOR PRE-SYMPTOMATIC DETECTION AND THERAPEUTIC TARGETING OF ALZHEIMER'S DISEASE AND DOWN SYNDROME IN HUMANS.

FIELD OF THE INVENTION:

The invention relates to highly specific diagnostic markers which are also highly specific targets for therapeutic reagents which are useful for detecting, preventing and treating Alzheimer's disease and Down Syndrome. More specifically, the invention relates to two unique proteins encoded on human chromosome 21, within the locus found to be occupied by human APP gene and believed to be occupied by gene/genes which are implicated in Down Syndrome, to analogues and derivatives of these molecules, and to nucleic acid molecules encoding such molecules or influencing their expression. The invention also relates to therapeutic methods for these molecules.

BACKGROUND OF THE INVENTION:

Alzheimer's disease ("AD") is a progressive disease of the human central nervous system. It is manifested by dementia in the elderly, by disorientation, loss of memory, difficulty with language, calculation, or visual-spacial skills, and by psychiatric manifestations. It is associated with degenerating neurons in several regions of the brain. All humans with AD develop a considerable number of ß amyloid plaques and

neurofibrillary tangles composed of highly phosphorylated forms of the microtubule associated protein tau; Alzheimer's disease is reviewed by Price, D.L. et al. (Clin. Neuropharm. 14:S9-S14 (1991)); Pollwein, P. et al. (Nucl. Acids Res. 20:63-68 (1992)); Regland, B. et al. (Med. Hypoth. 38:11-19 (1992)) and Johnson, S.A. (In: Review of Biological Research in Aging, Vol. 4., Rothstein, M. (Ed.), Wiley-Liss, NY, 163-170 (1990)).

The presence in postmortem brains of two proteins, hyperphosphorylated tau (PHF tau) and ß-amyloid (Aß), is the major pathological feature of all forms of human Alzheimer's disease (AD) and provides certain clues to some biochemical events in the disease. Normal tau is a cytoskeleton protein that functions as a microtubule "glue" in neurons, and ß-amyloid is a generally neurotoxic polypeptide fragment which is thought to be excised from the widely distributed APP transmembrane protein. PHF tau is present in the CSF of all humans with AD and Aß is present in CSF and blood platelets of people with AD. Another significant clue to physiological processes which contribute to the etiology of AD is the presence of numerous inflammatory proteins in characteristic Alzheimer's lesions.

Genes predisposing to AD.

Four genes and a protein, viz. the APP gene on chromosome #21 (St. George Hyslop, P. et al, Science 235:885-890 1987; Goate, A. M. et al, Nature 349:704-706 1991) S182 gene on chromosome #14 (Sherrington, R. et al. Nature 375:754-760 1995), the STM2 gene on chromosome #1 (Levy-Lahad, E. et al. Science 269:973-977 1995) and the ApoE4 gene on chromosome #19 (Saunders, A.M. et al., Neurology 43:1467-1472 1993) has been definitely implicated in the etiology of the disease. The neuron specific cytoskeletal protein "tau" which is secreted into spinal fluid, is highly phosphorylated in AD and the latter form is found in quantities, related to the stage of the disease, in spinal fluid taken from AD patients. Although the function of tau, in the initiation of AD is not obvious, a parallel can be drawn with the behaviour of

other microtubule proteins in epithelial cells which respond to transduction of membrane receptors by an unusual ligand. It has been shown that the interaction of enteropathogenic E.Coli (EPEC) within the membrane of cultured epithelial cells results in cytoskeleton rearrangement and hyperphosphorylation of three cytoskeletal proteins which migrate to a point below the site of membrane ligand interaction. The abnormally phosphorylated proteins form rigid aggregates (Rosenhine Llan, EMBO. J. 11: 3551-3560 (1992)) which are reminiscent of aggregates (NFTs) formed by tau in AD brains.

Mutations (several) in the APP gene segregate with an autosomal dominant, early onset form of AD in a few families (Goate, A. M. et al, Nature 349:704-706 1991; Selko, D. Scientific American 265:40-47 1991; Hardy, J. et al. WIPO WO 92/13069 1992) mutations in S182, segregate with familial AD [FAD] (St. George-Hyslop, P, et al. Nature Genet. 2: 330-334 (1992); Sherrington, R. et al. Nature 375:754-760 (1995)); a mutation in STM2 segregates with a rare autosomal form of AD (Levy-Lahad, E. et al. Science 269:973-977 1995), and inheritance of the ApoE4 allele appears to be a susceptibility factor in all the above mentioned forms of AD (Corder, E. H. et al. Science 261:921-924 1993), while inheritance of the ApoE2 allele appears to confer a decreased risk (Corder H. L. et al. Nature Genet.7:180-184 1994).

Nevertheless, the combined genetic factors account for only a small percentage of AD and, in spite of the identification of the latter factors, little is known or can be inferred about the biochemical cause of the disease symptoms. . About 80 % of all AD which occurs mainly in humans > 80 years old does not appear to be associated with genetic predisposition.

Genes predisposing to DS.

Trisomy 21, "Down syndrome" (DS) is a genetic disorder resulting from a chromosomal abnormality. It is one of the most common forms of mental disability and in addition affected individuals

frequently present a constellation of serious medical problems including susceptibility to rare forms of cancers (Shen, J.J., et al., AMJ. Human Genet. 56:915-925 1995), to heart and lung, hearing and visual, and motor problems. The vast majority of DS patients develop very early onset and severe Alzheimer's disease; however, the neuropathological changes in patients with DS do not parallel those of AD in all respects (Hof, P. R. et al., Arch. neurol. 52:379-391 1995). The disease affects 1.23/1000 children born to mothers over 35 years old (Carothers, A. D. et al.,Clin. Genet 46:405-409 (1994). In addition DS babies express Aß starting within a few weeks after birth. The genetic cause of the disease is believed to be duplication of a relatively small region of chromosome #21 which borders, on one side, the locus of the APP gene (Sinet, P. M. et al., Biomed Pharmacothr. 48:247-252 (1994); Querfurth, H. W., et al., Brain Res Mol Brain Res. 28:319-327 1995)).

The gene predisposing to susceptibility to AD and DS.

Inheritance of the ApoE4 allele also appears to lower the age of onset and increase the severity of AD in people with DS (Hyman, B. T. et al. Proc. Natl. Acad. Sci. USA 92:3586-3590 1995). The biochemical cause of DS is not known; there is no cure for the disease; however, genetic counselling has been able to cut the number of DS births significantly in some regions, and the presence of the genetic defect in unborn infants can be determined by an invasive procedure, "amniocentesis" and more recently by a noninvasive procedure, "The Triple Test". The test, which is based on the concentration of human chorionic gonadotropin, alpha fetoprotein and U3 oestriol in the blood of pregnant women, can be preformed on dried blood specimens (Verloes A, et al., Am J. Obstet Gynecol 172:167-174 (1995). However, although the Triple Test is a significant improvement over the invasive tests, it's accuracy requires both expert interpretation and exact knowledge of the gestational age of the foetus.

The gene products of genes predisposing to AD and DS

I. The APP gene on chromosome 21

The APP gene is preferentially expressed in the neuronal cells of the central nervous system. The gene encodes a trans-membrane protein which contains a single monomeric trans membrane alpha helix (Kang J. et al. Nature 325:733-736 (1987); Tanzi, R.E. et al. Nature 321:528-530 1988)). The protein, which is expressed primarily in brain, can exist as several isoforms of various length caused by alternative splicing or by incorporating or shedding exons (Pollwein, P. et al. (Nucl. Acids Res. 20:63-68 (1992); Price, D.L. et al., Clin. Neuropharm. 14:S9-S14 (1991)), a feature it appears to have in common with the LDL receptor protein. However, all the isoforms have a common domain called the ß Amyloid domain (Aß), that is partly embedded within the membrane spanning region of the APP protein. The major product of post translational cleavage of APP protein is Aß (Podlisny, M.B. et al., Science 238:669-671 (1987); Currie, J.R. et al., J. Neurosci. Res. 30:687-689 (1991)). (Zain, S.B. et al., Proc. Natl. Acad. Sci. (U.S.A.) 85:929-933 (1988); Vitek, M.P. et al., Molec. Brain Res. 4:121-131 (1988); Johnson, S.A. (In: Review of Biological Research in Aging, Vol. 4., Rothstein, M. (Ed.), Wiley-Liss, NY, 163-170 (1990)). The secretion of the Aß protein thus reflects the cleavage of the domain from the precursor molecule (see, Roch, J.M. et al., J. Biol. Chem. 267:2214-2221 (1992)).

AS indicated above the pathological hallmark of AD, and DS, is the presence of intracellular tangles, and extracellular deposits or "plaques" of amyloid protein Aß in the neuropil and in blood vessels (Mann, D.M.A. Neurobiol Aging 10:397-399 (1989); Selkoe, D.J. Neuron 6:487-498 (1991); Lampe, T.H. et al.Ann. Neurol. 36:368-378 (1994); Selkoe, D.J. Nature 375:734-735 (1995);). The principal component of the amyloid protein plaques in normal humans is Aß, a 38-40 amino acid (~4kDa) hydrophobic protein (Price, D.L. et al., Clin. Neuropharm. 14:S9-S14 (1991)). Aß

forms the core of fibrils, which are concentrated in amyloid deposits in the extracellular space of the brain parenchyma and in the vascular elements of the brain and the pia-arachnoid (Currie, J.R. et al., J. Neurosci. Res. 30:687-689 (1991)). However, in Alzheimer's disease a longer version of Aß, Aß-42 is released from APP and it is this species of Aß which is believed to be the major component of amyloid deposits and was also believed to be the neurotropic factor in AD (Roher A.E. et al.Proc Nat Acad Sci USA 90:10836-10840 (1993); Games, D. et al Nature 373:523-527 (1995); Laferia, F.M. et al., Nature Genet 9:21-30 (1995)), and in DS (Iwatsubo, T. et al., Ann. Neurol. 37:294-299 1995; Neuron 13:45-53 1994). Recently, it has been demonstrated in mice that the presence of a functional APP gene is not necessary for the formation of AD type neuropathology (Zengh, H., et al. Cell 81:525-531 1995), also that synaptic loss can occur by overexpressing APP without plaque formation (Mucke, L. et al. Brain Res. 666:151-167 (1994)).

Some of the above mentioned experimental findings appear to argue against a role for Aß in the neuropathology of AD and DS; nevertheless, the early build up of Aß in all forms of AD, (Querfurth, H. W. et al. Molec. Brain Res. 28:319-337 (1995); Levy-Lahad E., et al. Science 269:973-977 1995)), and the mutations in the APP gene which segregate with the disease, suggest a central role for APP in AD and DS (Hardy, John. Proc.Natl. Acad. Sci 94:2095-2097 (1997)).

The accumulation of Aß-42 in Alzheimer's disease is believed to result from the faulty processing of one or more of the APP isoforms (Currie, J.R. et al., J. Neurosci. Res. 30:687-689 (1991)), (see also, Johnson, S.A. In: Review of Biological Research in Aging, Vol. 4., Rothstein, M. (Ed.), Wiley-Liss, NY, 163-170 (1990); Roch, J.M. et al., J. Biol. Chem. 267:2214-2221 (1992)). Such processing is thought to involve two or more specific proteases (Azuma, T. et al., J. Biol. Chem. 267:1609-1613 (1992); Nakanishi N. et al., Exp Neurol 121:125- (1993)). At the moment there is a concerted effort by researchers to find

these proteases, ("secretases") essentially remain hypotethical to date; the method of Aß overproduction in AD and DS remains unknown.

II. Apolipoprotein E located on chromosome 19

The E4 allele of apolipoprotein, "ApoE4", originally associated with late onset familial and sporadic Alzheimer's disease (Saunders, A.M. et al., Neurology 43:1467-1472 (1993); Namba, Y. et al., Brain Res. 514:163-166 (1991)) appears to be associated with all forms of inherited HD and DS. The gene is widely expressed but the highest expression is found in brain.
ApoE4 is a member of a family of genes that also includes ApoE2 and ApoE3 (Utermann, G. et al., J. Lipid Res. 25:378-382 (1984); Utermann, G., J. Inher. Metab. Dis. 11:74-86 (1988); Ghiselli, G., et al., Lancet 2:405-407 (1982)). This gene family has been implicated in other serious human diseases involving faulty lipid metabolism, e.g., cardiovascular diseases. ApoE proteins bind to specific sites on the LDL receptor and on the related LRD receptor on cell membranes and serve as anchor sites for particular lipoproteins which are then transferred across the plasma membrane in coated pits and it has been shown that APP can bind to ApoE4 LDL receptors (Kounnas, M. Z. et al. Cell 82:331-340 1995) and hence may share a similar metabolic pathway; however, this gives little insight into how ApoE4 influences susceptibility to AD, or the staging and severity of AD in people afflicted with DS. ApoE proteins, like all soluble membrane proteins, aggregate in solution and ApoE4 is associated with Aß immunoreactivity in ß amyloid plaques. The latter might suggest that intra molecular protein aggregation could play a role in the pathogenesis of AD and DS. The finding that ApoE2 which has a cys residue at position 112, whereas ApoE4 does not, confers a decreased risk to AD to people having both alleles, suggest that a cys amino acid residue may confer the ApoE2 effect, and suggest that a cys residue may be involved in aggregation processes relevant to AD (Shi Du Yan et al., Nature 382:685-691 (1997); (Tabatin, M.et. al., Neurobiology of aging 17 (48) 5130 (1997))

III. S182 located on chromosome #14

Although the number of humans genetically predisposed to AD is relatively small, S182 is considered to be the major AD locus because the vast majority of Familial AD (FAD) patients have mutations in this region. The S182 gene encodes a transmembrane (TM) protein "AD3" which contains seven transmembrane helices. Five point mutations which occur in amino acid residues located in, or close to, different TM-helices, segregate with AD. Since the mutations were not found in normal humans they appear to be pathogenic for the AD phenotype in humans. The function of AD3 is unknown, but possible functional homology with SPE-4, a transmembrane protein expressed in the worm *Caenorhabditis elegans*, might suggest that AD3 can function in the cytoplasmic partitioning of proteins (Sherrington, R. et al. Nature 375:754-760 (1995); Hardy, John. Proc.Natl. Acad. Sci 94:2095-2097 (1997)).

IV. STM2 located on chromosome #1

Like S182, the STM2 gene encodes a TM protein that contains seven transmembrane helices. The STM2 protein is highly homologous to S182, especially to sequences within the TM helices. A single point mutation in amino acid (aa) 141 located in the region of the second TM helix, which is 84 % homologous to AD3 TM-II, was found to be the cause of AD in a large kindred family. As compared to FAD caused by mutations in S182, FAD caused by the STM2 141 mutation appears to be confined to one kindred, "The German Volga family". The structural similarity between AD3 and STM2 protein indicates that they share a similar biochemical function which is presently unknown (Levy-Lahad, E. et al. Science 269:973-977 1995). However the secondary structure of S182 and STM2 closely resembles that of the adrenergic and muscarinic receptors. In the latter, several transmembrane domains are required for determining selectivity of antagonist and agonist binding (Wess, J. et al Mol. Pharmacol. 256:872-877 (1991)). This might explain why any one of the 5 mutations which

occur in S182 can lead to AD.

Without some type of effective treatment, AD will probably affect about one out of every 10 humans alive today. Similarly, DS will continue to be the cause of mental retardation in a vast number of children, the majority of which will develop severe AD before the age of 50. There is no effective treatment for AD at any stage of its clinical progression and no reliable diagnostic method to detect the disease at early stages, even within the relatively small number of people genetically predisposed to the disease. There is no effective treatment for DS at any stage of the disease. Pre-natal diagnosis of the disease is possible but various human diversity-related factors, the requirement for expert interpretation and technical competence of the testing institutions makes accurate prediction difficult.

In view of the importance of diagnosing, predicting, and treating AD and DS, affective means for achieving these goals are pressing. The present invention supplies such means.

SUMMARY OF THE INVENTION

The object of the present invention was to provide highly specific molecules in humans which were highly reliable markers of AD and DS in humans. These molecules, in addition to being accurate predictive agents for the diseases, were also targets for in situ modification or destruction by therapeutic substances which would prevent and stop the progress of the diseases in humans without undesirable side effects.

According to the invention the highly specific molecules which are markers for AD and DS and also targets for therapeutic intervention have been provided.

The invention concerns agents and use of these agents as markers for early diagnosis and targets for therapeutic approaches for Alzheimer's disease and Down syndrome. Such agents include three

novel neuropeptides implicated in Alzheimer's disease and one novel neuropeptide implicated in Down syndrome (trisomy 21) as well as analogues and derivatives of these molecules, and nucleic acid molecules encoding such molecules, or influencing their expression.

BRIEF DESCRIPTION OF THE FIGURES:

Figure 1A. Nucleotide sequence of dsas cDNA, including the sequence surrounding the poly A addition site in the 3' untranslated region.
Figure 1B. Amino acid sequence of DSASp.
Figure 1C. Nucleotide sequence of 5' upstream regulatory region of dsas.
Figure 1D. Nucleotide sequence of in 5' upstream regulatory region of pac gene.
Figure 1E. Nucleotide sequence of pac cDNA.
Figure 1F. Amino acid sequence of PAC protein.
Figure 1G. Nucleotide sequence of alzas.
Figure 1H. (i) Amino acid sequence of ALZASp
(ii) Amino acid sequence of ALZASp3
(iii) Amino acid sequence of ALZASp4
Figure 1I. Nucleotide sequence of alzas1 cDNA
Figure 1J. Amino acid sequence of ALZASp1
Figure 1K. Nucleotide sequence of the 5' upstream regulatory region of alzas gene.
Figure 1L. Nucleotide sequence of alzas2 cDNA.
Figure 1M. (i) Amino acid sequence of ALZASp2
(ii) Amino acid sequence of ALZASp5
Figure 1N. Nucleotide sequence of the 5' regulatory region of alzas2 gene.
Figure 2A. Organisation of alzas gene, comparison with organization of APP gene: products expected from constitutive cleavage of ALZASp and from mutations in regions in chromosome# 21 encoding APP.
Figure 2B. Organisation of alzas1 gene, comparison with organization of APP gene: products expected from constitutive

cleavage of ALZASp1 and from mutations in regions in chromosome# 21 encoding APP.

Figure 2C. Organization of alzas2 gene, comparison with organization of APP gene: products expected from constitutive cleavage of ALZASp2 and from mutations in regions in chromosome #21 encoding APP gene.

Figure #D. Comparison of proteins "TRNAPP" and PAC" expressed by APP cDNA cloned into cells, with ALZASp and DSASp, respectively: products expected from constitutive cleavage of TRNAPP and PAC, in wild type and mutated APP mRNA.

Figure 3 A, 3B & and 3C. A Expression of AD and DS related mRNA in human brain and lymphocyte: (A) amplification with primer pair "alz289 - lane 1= DS brain, lane 2 & 3= normal brain, lane 4 = hippocampus, lane 5= AD cortex, (only a small amount of the PCR product was applied to the gel) lane 6 & 7= DNA size markers, lane 7= normal lymphocyte, lane 7= AD lymphocyte; amplification with primer pair "alz188"- lane 1 & 4= DNA size markers, lane 2= AD brain, lane 3 = normal brain; amplification with primer pair ds254, lane 1 & 2= normal trisomy 21 fibroblast cell line; lanes 3 & 4= DS fibroblast cell line, 5 & 6 = normal lymphocytes, 7 & 8 = DS lymphocytes; 9 & 10 = DS brains; 11-14 = normal brain 15 = DNA size markers.

Figure 4 A-B. ELISA methods

A. Description of the ELISA method used to detect ALZAS and DSAS and PAC in human body fluids and tissue.

B. Description of the ELISA method used to detect endogenous immunoglobulins produced in blood and serum of patients with Ad and DS.

C-E Detection of expression of AD and DS related proteins ALZASp (ALZAS), ALZASp1 (ALZAS1) and DSASp (DSAS) detection by immuno-chemiluminescent detection on dot blots.

For dot blots, proteins extracted from brain and lymphocyte by ammonium sulphate precipitation were dialysed and boiled for 5 minutes in 5% SDS. 2μl of the SDS treated proteins were spotted onto nylon membranes, treated with the appropriate antibody and detected with an immuno-chemiluminescent system: (A) probe = antibody ALZab2.

(C) dots were probed with antibody ALZab1; dots 1= normal lymphocytes, 2= DS brain, 3= DS lymphocytes, 4 & 5 = trisomy 21 fibroblast, 6 = normal brain, 7 = AD cortex, 8 & 9 = AD lymphocytes, 10 = normal fibroblast;

(D) 1.5 μl serum obtained from ten normal women over 35 years old selected at random was probed with ALZab1.

(E) detection of endogenous anti-DSAS IgG in serum and saliva od DS patients.

(F) Dot blots of serum from 28 autopsy confirmed AD victims was probed with ALZab2 (at least 50% of these patients we incorrectly diagnosed for a condition other than AD before death);

(G) Detecting different levels of ALZAS in serum from suspected early AD patients, patients with depression and autopsy confirmed AD victims.

(H) Detection of endogenous anti-ALZAS IgG in patients blood, serum and saliva

(I) western blots; 1 proteins in ammonium sulphate precipitates were electrophoresed on 17.5 % acrylamide gels, blotted onto nylon filters, reacted with the appropriate antibody and detected with the immuno-chemiluminescent system, 1. proteins were isolated from DS lymphocyte and treated with ALZab1; 2. proteins were isolated from AD hippocampus and treated with antibody ALZab2; 3. proteins were isolated from AD hippocampus and treated with ALZab3;

Figure 5A-D

(A). Isolation of ALZAS from serum obtained from a single autopsy confirmed AD patient. The protein was isolated using affinity purification methods on affinity purified anti ALZab2 antibody linked to CNBR-sepharose. The gels were stained with silver stains and western blotted. Human IG fragments were detected with mouse anti-human mABs.

(B) Silver stain of SDS gel: proteins obtained following affinity purification of ALZAS from serum from a late stage AD patient.

(C) Detection of ALZAS-IgG complexes present on gel of 5B with anti-human IgG (Fc fragment).

(D) Western blot of cationic non SDS polyacrylamide gel following electrophoresis of serum from (a) a patient with sporadic AD, and

(b) a patient with swedish mutation AD.

Figure 6 Alignments: (A) alignment of S182 TM-II, with ALZASpTM (APP-TM) and STM2 TM-2.

(B) alignment of c-terminal of ApoE4 with the c-terminal of ALZasp1

(C) alignment of the receptor/heparin binding site of ApoE proteins with the c-terminal of ALZASp1.

DETAILS OF THE INVENTION.

In detail, the invention provides a nucleic acid molecule, substantially free of natural contaminants, that encodes a protein selected from the group consisting of dsas, alzas, alzas1 and alzas2. In particular, the invention provides the above-described nucleic acid molecule wherein the sequence is SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:15.

SEQ ID:NO 1
5' ATGTCGGAATTCTGCATCCATCTTCACTTCAG
AGATCTCCTCCGTCTTGATATTTGTCAACCCAGAA
CCTGTATTACATCATAA 3'

SEQ ID:NO 11
5' ATGGATGCAGAATTCCGACATGACTCAGGATA
TGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAA
AGATGTGGGTTCAAACAAAGGTGCAATCATTGGAT
CATGGTGGGCGGTGTTGTCATAGCGACAGTGATCG
TCATCACCTTGGTGATGCTGAAGAAGAAACAGTAC
ACATCCATTCATCATGGTGTGGTGGAGGTAGGTAA
ACTTGACTGCATGTTTCCAAGTGGGAATTAA 3'

SEQ ID:NO 15
5' ATGGATGCAGAATTCCGACATGACTCAGGATA
TGAAGTTCATCATCAAAAATTGGTACGTAAAATAA
TTTACCTCTTTCCACTACTGTTTGTCTTGCCAAAT
GACCTATTAACTCTGGTTCATCCTGTGCTAGAAAT
CAAATTAAGGAAAAGATAA 3'

The invention also provides a protein, substantially free of natural contaminants, selected from the group consisting of DSASp, ALZASp, ALZASp1, ALZASp2. In particular, the invention provides the above-described protein having a sequence of SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:25. The invention also provides three associated hypothetical proteins having a

sequence of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:26.

SEQ ID:NO 3
M S E F C I H L H F R D L L R L D I C Q P R T C
I T S

SEQ ID:NO 12
M D A E F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I
I G L M V G G V V I A T V I V I T L V M L K K K Q Y T S I H H G
V V E V G K L D C M F P S G N

SEQ ID:NO 16
M D A E F R H D S G Y E V H H Q K L V R K I I Y L F P L L F V L
P N D L L T L V H P V L E I K L R K R

SEQ ID:NO 25
M V G G V V I A T V I V I T L V M L K K K Q Y T S
I H H G V V E V G K L D C M F P S G N

SEQ ID:NO 13
M Q N S D M T Q D M K F I I K N W C S L Q K M W V
Q T K V Q S L D S W W A V L S

SEQ ID:NO 14
M Q N S D M T Q D M K F I I K N W Y V K

SEQ ID:NO 26
M W V Q T K V Q S L D S W W A V L S

The invention also provides a reagent capable of diagnosing the presence of a molecule selected from the group consisting of dsas, a DSAS-encoding nucleic acid molecule, alzas, a ALZAS-encoding nucleic acid molecule, alzas1, a ALZAS1-encoding molecule, alzas2, a ALZAS2-encoding molecule.

The invention particularly concerns the embodiments wherein the reagent is a nucleic acid molecule produced from nucleic acid molecules having a sequence complementary to SEQ ID NO:1 (nucleotides 78-90), SEQ ID NO:11 (nucleotides 191-240), SEQ ID NO:15 (nucleotides 52-156) or SEQ ID NO:24 (nucleotides 96-170), or a nucleic acid molecule obtainable by mutating a nucleic acid molecule having a sequence of SEQ ID NO:1 (nucleotides 78-90), SEQ ID NO:11 (nucleotide 191-240), ID NO:15 (nucleotide 52-156) and SEQ ID NO:24 (nucleotide 96-170), or a protein (especially an antibody, or a fragment of an antibody, which is capable of binding to DSASp, ALZASp (amino acids 67-79), ALZASp1 (amino acid

18-51), ALZASp2 (amino acid 32-44), [hy1]ALZASp, [hy2]ALZASp and [hy]ALZASp2.

SEQ ID:NO 24
5' ATGTGGGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTG
TCATAGCGACAGTGATCGTCATCACCTTGGTGATGCTGAAGAAGAAACAGT

ACACATCCATTCATCATGGTGTGGTGGAGGTAGGTAAACTTGACTGCATGTTTCCAAGTGGG
AATTAA 3'

The invention also provides a method of treating Alzheimer's disease, and Down Syndrome by providing an individual, in need of such treatment, an effective amount of an antibody or anti peptide substance against DSASp, ALZASp, ALZASp1, ALZASp2, [hy1]ALZASp, [hy2]ALZASp and [hy]ALZASp2 or of a reagent to block the activation of the promoters (PDS1-PDS4 and PALZ1-PALZ14 and other regulatory elements which program transcription of dsas, alzas, alzas1, and alzas2 mRNA, or the heat shock elements which can influence activity of such promoters within the sequences identified as, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:17, SEQ ID NO:18; SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32.

SEQ ID:NO 2
5' GAAATAATAATAAAAGGCTTTAAAAAGAGCATTA
ATTACCTGGTAGAACAAAACTGCTACTTTGGGGCTT
TGTT 3'

SEQ ID:NO 4
5' AGGATTTTATATAACTACTAGAG 3'

SEQ ID:NO 5
5' GAATTTAAGCCACATAAAGTGTTCTGTAAAACCA
AGCAGTCTATG 3'

SEQ ID:NO 6
5' CCACTTTAAGTCCCGACTCAT 3'

SEQ ID:NO 7
5' CTGAAAGAACTATACATACAGCCTCTTTCTTTTCT

SEQ ID:NO 8
5' TAAATCATAAAACGGGTTTGTTTCTTCCCACATTA
TTCTATAAATTGCTGTGGCGGGGG 3'

SEQ ID:NO 17
5' TTGATAATTAAATGTTATAGCATGGACACTGACATT
TACATTTTTTACTTATGTTTTTGGTTTTTAAATGAC
TCTGCAT 3'

SEQ ID:NO 18
5' ATTATTATTTGAATAATGAAATTCATCAGAACAA
TTA 3'

SEQ ID:NO 19
5' GCAATTTATAGAAAAGGAAGAGTTCGTAGGTTA
TAAATTCTGTTAGTTGCTAAGAAGCATTTTTAAAA 3'

SEQ ID:NO 20
5' ATGCTCATTTTTAAAGGCTTTTATTATTATTTCT
GAAGTAATGAGTGCACATGGAAAAA 3'

SEQ ID:NO 21
5' TATTCCAGGAACAAATCCTTGCCAACCTCTCAA
CCAGG 3'

SEQ ID:NO 22
5' TAGCATGTATTTAAATGCAGCAGAAG 3'

SEQ ID:NO 23
5' GAAGGTTTAAATATAGGGTATCATTTTTCTTTA
AGAGTCATTTATCAATTTTCTTC 3'

SEQ ID:NO 27
5' CCAAATAAAGAGCAAGAATAAAGCAACATTTCA 3'

SEQ ID:NO 28
5' TTATGCTTTAAAAAGCAATACA 3'

SEQ ID:NO 29
5' TCCTTTCTTTCAGAATGCCTATTCCTGTGCATTA
AAAGTGTCCCTCC 3'

SEQ ID:NO 30
5' TTTAAAGTAAGCATCAAA 3'

SEQ ID:NO 31
5' CTTTTTATATAACCTCATCCAAATGTCCCCTGC
ATTTAA 3'

SEQ ID:NO 32
5' GAAAATGAAATTCTTCTAATTGCGTTTATAAA
TTGTAATTA 3'

DESCRIPTION OF THE PREFERRED EMBODIMENTS:

The consensus of all experimental evidence suggests: (1) that APP protein (the ß amyloid precursor) plays a major role in the

expression of AD disease phenotype regardless of origin of the initial biochemical trigger of the disease; the involvement of APP is manifested by the abnormal deposits of a variety of Aß molecules in crucial regions of the brain, (2) Aß is a 38-42, 4.6kd polypeptide which is derived from post-translational cellular processing of APP protein, i.e. it is a fragment of the ß amyloid precursor protein (see Selkoe, D.J. Neuron 6:487-498 (1991), Nature 375:734-735 (1995); Masters, C. L. et al. Proc. Natl. Acad. Sci. USA. 82:4245-4249 1985)) (3) the presence of Aß in brain and cerebral arteries signals the beginning of AD, although Aß does not cause the neurodegenerative symptoms characteristic of AD; (4) the APP gene is not overexpressed in AD although it is overexpressed in Down Syndrome because of the additional gene dosage, but the same might apply to other chromosome 21 associated genes that have no involvement in DS, (5) the emphasis for finding the biochemical cause of AD has shifted to finding the proteases responsible for faulty cellular processing of APP and, hopefully, targeting these for diagnostic and therapeutic, finally (6) in the meantime, there is neither a reliable non invasive method to detect AD in the early stages nor an effective method to treat the disease.

The success of the present invention came mainly from our realization that there must be other genes within the APP locus with association to the AD and DS, and that at least one of these genes must also have a ß-amyloid related component. Therefore, we used a procedure which we had successfully used to find alternative genes, which are putative causative factors of other "genetic diseases", to search for such genes which might segregate with AD and DS, within the locus encoding the entire APP gene on chromosome 21 and the regions that flank the gene.

We call these alternative genes "piggy-back genes", and we refer to this technology as "disease gene discovery by positional searching" (DGDPS). Piggy genes are transcribed in any orientation within the chromosomal locus occupied by another gene.

DGDPS procedure.

This procedure has an advantage over gene isolation by cloning from a genomic or cDNA library, because it overcomes three important drawbacks, (1) the possibility that some DNA sequences cannot be cloned by the conventional methods, (2) that some mRNA sequences are of such low abundance that they are not represented in the cDNA library, and (3) the products of some cloned sequences are highly toxic to bacterial or other hosts.

In general, first we identified a gene closely related to a gene already genetically linked to a certain disease, then isolated the mRNA transcribed from the gene from disease tissue or patient's blood, then synthesized cDNA from the isolated mRNA with reverse transcriptase then amplified the novel cDNA with specific primers which flanked the entire coding region of the cDNA, then we identified the cDNA from the size following electrophoresis on agarose gel, and finally isolated the unique cDNA from the agarose gel. This allowed us to select out the desired molecule, if it was expressed, without having to probe several million cDNA clones.

The fact that Aß, which is present in all AD, is a part of the APP protein, and other indications from results of our work with some other neurodegenerative diseases, we concluded that the biochemical link between all forms of AD was the APP protein. We hypothesized that Aß was not directly generated from APP embedded in the membrane, rather it came either from "soluble" i.e., transported APP or from a different protein which was likely to be expressed in significant amounts in a disease specific manner. We called the protein "ALZAS" (Alzheimer's disease associated), and we predicted that ALZAS would be structurally closely related to APP and transcribed from within the same chromosomal location as APP. Furthermore we predicted that ALZAS would influence the phosphorylation of tau, would initiate the immunological reactions which lead to complement formation in neurons and ALSASp would be present in body fluids (serum, saliva, urine) of

people with AD. The aim of this project was to find ALZAS in patients with AD.

The present invention derives in part from the discovery and cloning of 4 (four) novel genes "alzas", "alzas1", "alzas2" and "dsas" using the above approach. Three genes are involved in the etiology of all types of Alzheimer's disease, and the fourth, dsas, in the etiology of Down Syndrome. Following is a detailed description of positional searching as it applied to the present invention:

(1) We examined the sequenced regions within the APP locus on chromosome 21 and selected potential complete orf's, i.e. with acceptable translation initiation sequences (see Kozak, M. Nucleic Acid Res. 12:857-872 1984) and translation termination stop codons (TAA, TAG or TGA) in place,
(2) next we used the method of Bucher et al., J. Mol. Biol. 212; 563 - 578 (1990) to identify putative promoter regions associated with the orf within 100-1000 bp 5' upstream of the translation initiation sequence and we identified potential poly-A addition signals (the consensus poly-A addition sequence is AATAAA) within a region of ~1000 bp 3' downstream from the stop translation codon of the potential orf,
(3) then, orf's fulfilling the above two characteristics were translated into putative protein sequences using the universal code,
(4) then we analyzed the putative protein with our proprietary computer assisted protein finger printing technology and obtained information about the potential biochemical characteristics of the deduced proteins,
(5) next the biochemical characteristics of the deduced proteins were correlated with known clinical symptoms of the AD and DS (Mann, D.M.A Neurobiol Ageing 10:397-399 1989), and with the biochemical characteristics of the disease reported (Selkoe, D.J. Neuron.6:487-498 1991),
(6) RNA encoding proteins with properties correlating with the disease characteristics were selected as potential disease

related candidates,

(7) detection of the presence of transcribed mRNA sequences encoding the protein in a cell was done by PCR, (Mullis, K.B., Cold Spring Harbour Symp. Quant. Biol. 51:263-273 (1986); Saiki, R.K., et al., Bio/Technology 3:1008-1012 (1985); Mullis K. et al., U.S. Patent 4,683,202; Erlich, H., U.S. Patent 4,582,788; Saiki, R. et al., US 4,683,194 and Mullis, K.B., et al., Met. Enzymol. 155:335-350 (1987); in detail reverse transcriptase-PCR (RT-PCR) was done using the Stratagene RAP-PCR RT-PCR kit according to the manufacturer's instructions, with unlabelled primers to detect cDNAs encoding the deduced proteins in RNA isolated from frozen human brain and lymphocytes.

RNA from frozen brains were extracted by grinding postmortem frozen brains in a tissue homogenizer in the presence of diethylpyrocarbonate (DEPC). Total RNA was isolated using the Stratagene micro RNA isolating Kit and poly (A)+ was isolated using Stratagene Poly(A)+ Quick mRNA isolation kit following the manufacturer's instruction. Isolated RNA was treated with 10 units of RNAse free DNAse at 37oC for 15 minutes. DNAse was inactivated by treating for 2 mins at 100 oC. cDNA synthesis using mRNA as template was carried out with the first strand protocol supplied with the and RT-PCR was done using the cycling conditions recommended by the manufacturer. Forward and reverse PCR primers were prepared to regions flanking the entire protein coding region of the orf of the selected protein (see table 1 for sequence of the primers and for the size of the expected amplified product). The amplified cDNA was electrophoresed on agarose gels and the size was determined by comparison with DNA size markers which were electrophoresed alongside. To verify the sequence of the cDNA, the region of agarose containing the desired size cDNA was extracted into H2O, precipitated with ethanol and a portion was cycle sequenced using the primers in "12" and Perkin Elmer ampli-Taq on the Perkin Elmer 376 A DNA sequencer using a non radioactive method described by Liu, C. et al.Nucl. Acid. Res 21:333-334 (1993).

(8) To determine if the proteins were actually expressed, epitopes were identified within the amino acid sequence of the

protein using the method of Hopp, T.P. and Woods, K.R. Proc. Natl Acad Sci. USA 78:3824-3828 (1981) and their sequences compared to sequences in databases; epitopes (see table #2) having no homologue within the public databases were selected and mono-specific polyclonal rabbit antibodies were prepared against these and purified by immunoaffinity chromatography on Pharmacia LKB, CNBr-activated sepharose 4B according to the recommendation of the manufacturer (see section #11 on Immunology, in Current Protocols in Molecular Biology (Vol 1) Ausubel, F.M. et al (ed) John Wiley & Sons NY. NY. 1991).

9. To detect the expression of the deduced proteins in human material, proteins were isolated from AD, DS and normal tissue, by precipitating the homogenized tissue with >80% ammonium sulphate and dialysis against SDSPAGE buffer (the buffer conditions are described in Laemmeli, U.K. Nature 227:680-685 (1970)), (16) dialysed proteins were boiled in SDSPAGE buffer and either electrophoresed in 17.5% acrylamide gel, following which the proteins were Western blotted onto nylon membrane and treated with the affinity purified antibody or spotted onto positively charged membranes and treated as above. Interaction of the antibody with the protein bound to the membrane was visualized with a chemiluminescent kit purchased from BioRad Inc according to the manufacturer's instructions (also see Blake M.S. et al.Anal. biochem. 136:175-179 (1984).

ALZAS has structural similarities to small pore-forming proteins. Pore-forming proteins found in a variety of organisms penetrate cellular membranes and mediate membrane damage, usually at a very low effector/target cell ratio. For a pore forming protein to be effective, two interconvertible configurations of the protein must exist in the cell: (i) for membrane insertion the protein must present a hydrophobic domain on the surface which must not be dominant, (ii) whereas for traversing the cytoplasm to reach the membrane the protein must behave as a soluble entity. The interconvertible configurations might be achieved in several ways including oligomerization, or interaction through hydrophobic

sites with a chaperon. In order to enter the membrane, on contact with the membrane dissociation of the oligomer occurs which re-exposes the protein hydrophobic domain and drives the membrane insertion. ALZAS has the APP transmembrane signal; therefore, like APP, ALZAS can be translocated across the membrane of the endoplasmic reticulum (ER) and the cytoplasm and can insert into the same sites as APP in plasma membranes.

The genes alzas, alzas1, alzas2 and dsas, and the protein encoded by these genes are described in the examples given below. Our discovery that these molecules: (i) were expressed in (100%) of brains, lymphocytes and blood obtained from humans with AD or DS respectively, (ii) elicited an antibody response in humans before clinical symptoms of the disease was detected, (iii) were not detected in normal individuals (normal = individuals below 30 years and individuals above 60 with no history of a neurodegenerative disease), and (iv) in accordance with the known incidence of AD in the population, were detected in 2 of 5 clinically normal people over the age of 65 who appear suspect for AD. Strongly supported involvement in the etiology of AD and DS. Furthermore it was a strong indication that these molecules could be used for presymptomatic diagnosis of AD, and DS, and furthermore were targets for active and/or passive vaccines type therapeutics to prevent and cure AD and DS.

THE MOLECULES OF THE INVENTION

Example 1

1. The **DSASp DNA binding neuropeptide**.

The invention relates to the discovery of a gene which we call Down Syndrome Related ("dsas"). dsas was selected following steps 1-8 of the procedure described above. dsas is encoded within the locus of the APP gene on human chromosome 21. The positional relationship of dsas to the APP gene is shown in the embodiment of figure 2A. Transcription of dsas is programmed by, at least,

four promoter systems, ("PDS1") SEQ ID:NO 4, ("PDS2") SEQ ID:NO 5 , ("PDS3") SEQ ID:NO 6 and ("PDS4") SEQ ID:NO 7. PDS2/3 & 4 are correlated (a promoter system is said to be correlated when it contains a TATA box and cap site or CCAAT box or GC box located within a defined distance from each other in the 5' upstream regulatory region of a gene). The transcriptional regulator region of dsas is shown in the embodiment of figure 1 C within a sequence of 500 nucleotides, in a region complementary to sequences in APP gene intron 16. As shown in figure 1A dsas gene harbours a consensus poly-A addition site (SEQ ID:NO 2) which starts at +386 relative to the orf stop translation codon in the 3' downstream untranslated region. This region of dsas gene is complementary to a region in intron 15 of the APP gene.

dsas cDNA, SEQ ID:NO 1 is shown in figure 1C. As shown in figure 2A the sequence is complementary to sequences in APP gene intron 15 and exon 16. The sequence of the protein encoded by dsas (DSASp) SEQ ID:NO 3, is shown in figure 1D. The protein harbours a putative zinc finger motif and a putative farnesyl group binding site. Fingerprinting analysis indicated that DSASp is a DNA binding neuropeptide with a wide variety of potential pathogenic activities, e.g., it can influence or mimic the activity of the human embryonic growth factor, the ß lymphocyte activation antigen, the meiotic recombination protein MER-1 which is involved in chromosome pairing, the interferon induced GTP-binding protein and the apoptosis regulator BCL2 homologue. It has a protease-like domain which can influence the activity or mimic pathogenic proteases, including viper Russelli proteinase, and a killer toxin with carboxypeptidase-like activity. DSASp can also mimic or influence the sperm DNA binding "protamine Z1" protein. The latter protein forms highly condensed, compacted, chromatin packets consisting of membranes and chromosomal DNA in sperm heads. DSASp can modulate the activity of the serum retinol binding protein (in plasma, 40% of the retinol binding protein is bound to transthyretin which is strongly implicated in hereditary amyloidosis).

Detection of transcribed dsas.

RNA from DS and normal people was prepared and cDNA synthesis of RNA were done as described above. PCR primer set ("pp") ds254 (table 1a) was used to amplify dsas specific cDNA as described above. The expected size of the amplified cDNA fragment was 254 bp which included the entire protein coding region for DSASp. As shown in figure 3B, lane 3-4,and 7-10, the fragment of the desired length, 254 bp, was obtained when cDNA prepared from DS fibroblast, DS lymphocytes and DS brains were amplified . No amplification when the corresponding normal tissue was used in the amplification process. The amplified cDNA band was eluted from the agarose gels and cycle sequenced as described above. In every case the nucleotide sequence was identical to the predicted sequence which included the sequence SEQ ID:NO 1 (data not shown).

Detection of DSASp.

Crude proteins isolates prepared from blood and brain of DS, AD, normal persons, and fibroblast cell lines as described above, were probed with antibody ALZab1 (table 1b). As shown is figure 4C, DSASp was detected in DS brains (dot 2), DS blood (dot 3) trisomy 21 fibroblast (dots 4 & %), but not in normal fibroblast (dot 10), normal lymphocytes (dot 1), normal brain (dot 6), AD cortex (dot 7) AD lymphocyte (dot 8); lymphocytes from one AD patient had a significant reaction with the antibody (spot 9). Blood and saliva from DS patients were similarly investigated for the presence of anti-DSAS endogenous antibody, figure 4D the antibody was found in the five patients tested.
Western blots probed with ALZab1 showed a immunoreactive band at the expected size, 3.3 kd, for DSASp, figure 4D lane 1.

The above results were confirmed by test in brain and blood from twenty normal humans, thirteen AD victims, seven DS victims and three trisomy 21 fibroblast cell lines. Therefore, according to this invention it is possible to use PCR primers and

antibodies against DSASp to detect, with very high level of confidence, DS in humans cells bearing trisomy 21. Since DSASp has the biological properties to cause, at least, some of the symptoms of DS, eliminating the molecule can lead to cessation of the symptoms; therefore, DSASp and dsas are also targets for therapeutic reagents to stop DS in humans.

As seen in figure 4c, it is possible to detect DSASp with the antibody in serum of some women who are clinically normal and do not have clinical symptoms of Down syndrome. This suggest that the protein might be present in women prior to conception and, perhaps these are the women who conceive a Down syndrome baby.

In addition, according to this invention, the combination of non expression in normal cells and the complexity of the promoter system located in the 5' regulatory region of dsas gene is an indication that the promoters can be regulated by a number of factors which are not normally present in the cell. Preventing activation of the promoters is, therefore, an ideal method to prevent expression of dsas mRNA, which would stop and prevent symptoms of DS and DS related AD.

In summary, by mimicking protamine Z1, DSASp, in a tissue specific manner, may bind to and condense chromosomal DNA in local regions and influence the expression/overexpression of a number of critical genes, especially early developmental genes and probably alzas, the gene that encodes ALZASp.

Example 2

The ALZAS transmembrane protein.

The invention also relates to a gene which we call Alzheimer's associated ("alzas"). alzas is encoded within the APP locus on chromosome 21. The positional relationship to APP encoding sequences is shown in figure 2Ai. The gene comprises two exons separated by a 5.6 kb intron. The nucleotide sequence of the transcription regulatory region of alzas, which lies within intron 15 of the APP gene, is shown in Figure 1 K. Transcription

of alzas can be programmed by any of eight promoters, ("PALZ1")+("PALZ2") SEQ ID:NO 17; ("PALZ3") SEQ ID:NO 18, ("PALZ4") SEQ ID:NO 19, ("PALZ5") SEQ ID:NO 10, ("PALZ6") SEQ ID:NO 21, ("PALZ7") SEQ ID:NO 22 and("PALZ8") SEQ ID:NO 23, located in the regulatory region. PALZ3/4/5/6/7/8 are correlated with cap sites. Heat shock elements, which can modulate activity of all the alzas promoters, overlaps PAL3 (Heat shock proteins are reviewed by Gething, M.J. and Sambrock, J. Nature 355:33-45 (1992); two putative estrogen responsive elements (Savouret et al., Recent Progress in Hormone Res. 45:69-120 1989; Beato M. Cell 56:355-361 1989), lie ahead of and between PALZ 7/8. A potential poly-A addition site is present in the 3' downstream untranslated region of the gene.

The alzas cDNA is shown in figure 1G SEQ ID:NO 11. It includes sequences identical to APP exon 16, exon 17 and part of intron 17, and encodes a 79 amino acid protein ALZASp, SEQ ID:NO 12, shown in figure 1 Hi. The protein includes the complete Aß protein sequence, the APP transmembrane helix sequence (which has a constitutive hormone controlled secretory signal between aa 42//43) and a unique c-terminal sequence that is not related to APP amino acid sequences but has significant homology to a domain in a plant chloroplast membrane protein, and to the c-terminal sequence of ApoE4 proteins (see figure 6b) as determined with the method described by Feng, D.F. et al. J. Mol. Evol. 21:112-125 (1985).

How ALZASp might cause or initiate AD in humans.

The presence of a transmembrane helix (TM) identical to APP TM means that ALZASp (which is expected to have a far less complex secondary structure than APP because of size) can successfully compete with APP for membrane anchor sites and may prevent APP from inserting into membranes. When this happens APP protein becomes vulnerable to degrading lysosomal protease activity. The latter could generate increased levels and various sizes of Aß protein fragments without any change in the actual level of APP

mRNA transcription and APP protein expression in cells.

Computer assisted analysis of the secondary structure of ALZASp revealed four tandem amphipathic alpha helical regions. This helical organization, including the transmembrane helix, suggested that ALZASp might be related to membrane pore-forming proteins. Characterized pore-forming proteins include, cytotoxic lymphocyte perforin, human complement C9 (Peitsch,M et al.Mol. Immunol 27:589-602 1990), and human defensins. All membrane penetrating peptides appear to use the amphipathic alpha helix (Hill, H.P. et al. Science 251:1481-1485 1991). In order to remain soluble during cellular transport to membranes, a pore forming protein must not have an exposed hydrophobic surface. This can be accomplished by folding or by oligomerization of the molecule which buries the hydrophobic regions while exposing a hydrophillic surface. On contact with the membrane the oligomers dissociate or unfolds and the hydrophobic domain drive the membrane insertion (Hill, H.P. et al 1991 ibid). By punching holes in a membrane, pore forming proteins cause slow dissolution of the membrane. This leads to increasing disruption, and eventual loss of function, of membrane proteins in the vicinity of the pore.

Stable interaction with another cellular protein can prevent oligomerization of a pore forming protein, and may prevent burial of the pore forming hydrophobic surface; the latter will have the effect of keeping the pore forming protein in the insoluble state; a condition which can effectively prevent the protein from reaching the membrane. In this regard, the evolutionary relationship between the c-terminal domains of ApoE proteins and ALZAsp, indicated above, might be important. ApoE/2/3 might prevent oligomerization of ALZASp by interacting with and forming an intra-molecular cys-cys bridge between, ApoE cys 112 and ALZASp, cys 73, in the c-terminal domain. ApoE4 cannot prevent ALZASp oligomerization because it lacks the cys 112 residue. This mechanism is consistent with the relative effects of a double and single dose of ApoE4 allele on the chances of getting AD.

By comparison with other multi-ligand, ligand-binding receptors (e.g. ß- adrenergic receptors, tachykinin receptors) AD3 and STM2 appear to be receptor sub-types. In these receptors the structural integrity of each TM helix is required for proper interaction between ligand and receptor (it has been demonstrated that several transmembrane domains are necessary to form the ligand binding pocket required for determining the selectivity of binding of ligands to adrenergic and muscarinic receptors (Wess J. et al. Mol. Pharmacol. 38:872-877 (1990)). This is one explanation for how mutations within or close to five TMs in AD3, and close to TM-II in STM2, are pathogenic in AD.

Structural and functional considerations indicate that ALZAS in addition to the potential to mimic and replace APP in transmembrane transport and interactions might also compete for TM sites with PS1/PS2. The core region of ALZASp/(APP)-TM has significant evolutionary relationship with AD3 TM-II and STM2 TM-II. ALZASp might compete successfully with S182 and STM2 proteins, in which mutations have altered the structural integrity of the TM, for TM sites in intracellular membranes. This could increase the competitive advantage of ALZAS for blocking the inter/intramembrame interaction between APP and PS1/PS2 ((Citron, M. et al., Nature Medicine 3: 67-72 (1997); Lamb Bruce. T. Nature Medicine 3:28-29 (1997)). Leading to a faster and greater exclusion of APP from intracellular vesicles leaving this molecule to the mercy of all types of proteolytic activity in the cytoplasm.

Furthermore affinity purification of ALZAS on columns of anti-ALZAS-sepharose columns of SDAT patients indicate that the ALZAS protein is bound to human immunoglobulin fragments in AD patients. This indicates that ALZAS is modulated by the immune systems in AD victims, and may be a target for complement derived destruction (see McGeer P.L. and McGeer, E.G.Ann NY Acad. Sci. 777:213-220 1996). The structural similarity between ALZAS and APP means that complement or other proteases directed at ALZAS

may also inadvertently target at Aß and the transmembrane sequence in APP protein. Joint proteolytic targeting of APP and ALZAS could explain all forms of Aß and can furthermore explain how Aß appears to, be cut in a portion of the APP which lies within the TM helix. This also obviates a functional significance for specific digestion of APP by unique proteases. Therefore, it appears likely that the condition of a human immune response might play a significant role as to who gets AD and it makes the role of inflammation in the etiology self explanatory.. It is possible that disruption of cytoskeletal structure which leads to abnormal phosphorylation of tau by activated tyrosine kinases a direct result of ALZAS interaction with the neuron plasma membrane (See Knulton J. et al., Infect. and Immuno.,57:1290-1298 (1989), Rosenshine et al., EMBO J. 11:3551-3560 1992)) in the same manner as production of multiple forms of Aß is due to the exclusion of APP from the membrane by competitive ALZAS binding and subsequent digestion by proteolytic enzymes.

Given the above indications, it appears that DS and AD may not be as closely related as we think. The factor which connects the two diseases i.e., the presence of Aß, which may have little physiological significance in AD and DS is likely caused by two opposite mechanisms each resulting in production of Aß from free APP (i.e. associated with the membrane). In DS the accumulation of Aß is due to the overproduction of APP, which over-saturates membrane sites and leads to exclusion of a significant amount of APP molecules, which are digested in the cytoplasm; in AD, APP is out-competed for membrane sites by ALZAS and remains in the cytoplasm where it is digested.

Amplification of alzas in human frozen brains and lymphocytes

RNA was isolated from 13 frozen normal human brains and lymphocytes, frozen AD brains and lymphocytes. cDNA synthesis of mRNAs were done as described above and cDNA amplification was carried out with pp alz287 (see table 1a) (and with pp alz393 data not shown) which was expected to amplify a 287bp fragment.

The amplified fragment was detected by comparison with DNA size markers electrophoresed under the same conditions (figure 3A). Lane DS brain, lane 2 & 3 normal brain, lane 4, AD hippocampus, lane 5, AD cortex, lane 6 & 9 DNA size markers (Boheringer, marker #5), lane 7, normal lymphocyte, lane 8 AD lymphocyte. The amplified 287 base pair fragment was isolated from the agarose gel and subjected to DNA cycle sequencing using the nonradioactive method described previously for pp 287. The results (not shown) matched exactly the predicted nucleotide sequence which included, SEQ ID:NO 11.

Detection of ALZASp in frozen human brains and lymphocytes

Aliquots of proteins isolated, as described above, from frozen human brain, frozen AD brain, normal lymphocytes and AD lymphocytes, were spotted on a nylon membrane or subjected to western blotting as described, and probed with antibody ALZab2. ALZab2 detects only the last 12 amino acids in the c-terminal of ALZASp. The results in figure 4E &F show the presence of ALZASp in AD cortex and lymphocyte respectively.
Western blots, figure 4D, lane 2 probed with ALZab2 showed a single, immunoreactive, band of 8.6 kd which was the expected size of ALZASp.

The presence of multiple promoter elements, heat shock elements, and putative estrogen receptor elements in the regulatory region of alzas is an indication that transcription of alzas mRNA might be triggered by a number of different factors including external environmental factors unrelated to mutations, e.g., stress inducing factors, metals, etc. However, certain factors might have a suppressive effect on the activation of the promoters (e.g estrogens, nicotinic acid related molecules, estrogens) and hence may delay, or even prevent, AD in some humans. Therefore, according to this invention these promoters are ideal targets for therapeutics which will block activation and prevent transcription of alzas in humans.

Example 3

The ALZASp1 membrane associated transcription factor.

This invention also relates to a gene we call Alzheimer associated 1 ("alzas1"). The location and organisation of alzas1 with respect to alzas is shown figure 1 I. The gene comprises an extended alzas exon 1. It shares the same 5' regulatory sequences as alzas but it terminates within sequences homologous to APP intron 16. It has a consensus poly A site in the 3' untranslated region.

The alzas1 cDNA, SEQ ID:NO 15, is shown in figure 1 J. It encodes a 51 amino acid protein "ALZASp1" figure 1 K, SEQ ID:NO 16. ALZASp1 is made up from the first 17 amino acids of Aß and 34 aa encoded by sequence homologous to APP intron 16. The protein contains a monomeric transmembrane helix and has a classic "leucine zipper"; it has a secretory signal which, when used, releases the entire non membrane associated c-terminal domain. As shown in figure 6c, the c-terminal of ALZASp1 contain a five amino acid sequence which is identical to ApoE proteins heparin binding site and to the core sequence of ApoE LDL receptor binding sequence. This suggests that ALZAS1p might compete with ApoE proteins for binding to the LDL receptor and may be an important etiological factor in AD associated vascular diseases. It also provides additional indication that an evolutionary relationship might exist between ApoE proteins and ALZAS proteins. ALZASp1 is a plasma membrane associated transcription factor. Our fingerprinting analysis indicated that the protein is related to ADR6 the important yeast transcriptional activator which activate many genes, and the yeast longevity assurance protein. Also, it might have the potential to modulate or mimic human delta type opiate receptor, the somatotropin receptor, the melanocyte stimulating hormone receptor, leucocyte surface antigen, sterol-o-acetyl transferase, the brain glutamate/aspartate transporter and the high affinity IgG Fc receptor.

Amplification of alsas1 in human frozen brains and lymphocytes.

PCR was done with pp alz188 using the same cDNA preparations from which alzas was amplified. The expected size of the amplified cDNA was 188bp. significant amounts of the expected size cDNA was amplified from AD hippocampus figure 3C lane 2, whereas, considerable less cDNA was amplified from normal brain, lane 3 or from normal lymphocytes and normal brains, results not shown. Similar results were obtained by amplification with pp alz 267 (see table 1a). Cycle sequencing of pp alz 267 amplified cDNA isolated from agarose gels provided a nucleotide sequence identical to the predicted cDNA sequence which included SEQ ID:NO 15.

Detection of ALZASp1 cDNA in frozen human brains.

Aliquots of protein isolated from frozen normal and AD brains (cortex) were spotted on nylon membrane as described above for DSASp and probed with ALZab3 (see table 1b). Immunopositive reactions were obtained with both normal and AD proteins but the reaction of the AD proteins, was considerably greater than that of the normal brain, AD lymphocytes and normal lymphocytes. Western blots done with of a crude preparation of ALZASp carried out as described before, showed an immunoreactive band at 6.15 kd, figure 4G, which was the predicted size of ALZAS1p.

According to this invention alzas1 is overexpressed in AD and alzas is expressed only in AD. Since these genes share the same 5'upstream regulatory region, the signal that activates promoters transcribing alzas must also upregulate alzas1 promoter/s. ALZAS1p can prevent Apoe4 from interacting with LDL type receptors; ApoE plays a chaperon role in mediating entry of APP (with which it might share a receptor binding site) through the plasma membrane. Therefore, APP entry into cells may be impeded by ALZASp1 compounding the effect of ALZASp as proposed above. Thus, ALZASp1 is a potential target for therapeutics which might contribute to preventing and stopping AD in humans.

Example 4

The ALZAS2p membrane associated protein.

The invention is also related to the discovery of a gene we call Alzheimer associated 2 ("alzas2"). The organization of the gene in relationship to alzas is shown in figure 2 C. It is a single exon gene that is formed from an extended version of alzas exon 2. It is transcribed by six promoter elements ("PALZ9") SEQ ID:NO 27, ("PALZ10") SEQ ID:NO 28 ("PALZ11") SEQ ID:NO 29, ("PALZ 12") SEQ ID:NO 30, ("PALZ13") SEQ ID:NO 31 and ("PALZ14") SEQ ID:NO 32, located in the 5' upstream regulatory region of the gene. The sequence shown of the latter region is shown in figure 1N, it is homologous to sequences in APP intron 16. It harbours two potential heat shock elements, one upstream of PALZ11 and the other upstream of PALZ14, which may influence activity of the promoters.

The nucleotide sequence of alzas2 cDNA, SEQ ID:NO 24, is shown in figure 1 L. There are two potential orfs in the cDNA; the most probable orf encodes a 44 amino acid protein ALZAS2p, SEQ ID:NO 25, figure 1 Mi. The aa sequence of ALZAS2p is identical to sequence 36 - 79 of ALZASp. It contains 80% of ALSASp TM sequence and the unique c-terminal domain, which has homology to ApoE proteins c-terminal domain. However, the truncated TM sequence, which according to our analysis has the potential to enter the membrane, in ALZAS2p is located directly at the n-terminal domain of ALZAS2p. With respect to competing with APP for membrane anchor sites, and intermolecular aggregation with ApoE proteins, ALZAS2p is expected to act in a parallel manner to ALZASp. The other hypotethical protein ("[hyp]ALZAS2p") SEQ ID:NO 26, which can be translated from alzas cDNA is shown in figure 1 Mii; protein fingerprinting indicates it is a neuropeptide with a wide spectrum of physiological activity.

Amplification of alzas2 cDNA in frozen brains and lymphocytes.

pp alz141 which flanks the entire coding region of alzas2 was used to amplify the same preparations of cDNA used with pp alz287 No cDNA was amplified from DS lymphocytes, from AD lymphocyte, or from AD brain. cDNA was not amplified from five normal brain we tested. The failure to find transcripts for alzas2 in AD brains and lymphocytes is consistent with our expectation that alzas2 is transcribed only in those instances where FAD involves the DM to QL mutation in APP exon 16. ALZAS2, not ALZAS, is expressed in humans with this mutation (Swedish mutation) which is believed to be pathogenic for AD. The following two examples demonstrate that ALZAS2, not ALZAS, is involved in the pathology of this variety of FAD since ALZAS cannot be expressed in humans with this mutation, and ALZAS2 share the same antigenic site to which anti-ALZASa1b/a2b are directed.

Example 1: Detecting ALZAS protein in serum from people with the Swedish mutation using the ELISA procedure as in figure 4A. In figure 5 a, subjects 1-5 have no mutation, subject 6 has the mutation and has clinical AD, subjects 7-10 have the mutation but no clinical signs of AD. As can be seen, patients no 6 and no 9 had high levels of ALZAS.

In Example 2, figure 5 b, serum from a patient with sporadic AD, post mortem confirmed, and patient no. 6 from example 1, was subjected to cationic SDS-free polyacrylamide gel electrophoresis, western blotting and immuno-reacted with anti-ALZAS b. Positive reacting bands were visualized by subsequent treatment with chemiluminescence anti-rabbit IgG system. As can be seen, that ALZAS reacted with protein bands in both, the serum from sporadic AD and from the Swedish mutation AD. It can also be seen that the antibody interacted with a complex which we have found in other experiments, to be a complex of ALZAS and endogenous human anti-ALZAS IgG. In addition to demonstrating that ALZAS2 is a factor in the Swedish mutation AD, the experiment suggests that (i) ALZAS2 can be detected in humans presymptomatic for AD, and that (ii) only the transmembrane helix and the (intron encoded) unique c-terminal piece of ALZAS protein is required for AD.

In summary, ALZAS2p when expressed can mimic some of the biochemical activities of ALSASp which is involved in AD.

Effect of mutations in APP exons 16 & 17 on the expression of ALZAS proteins.

There is a remote possibility that the region of chromosome 21 where alzas, alsas1 and alzas2 are located may be a duplicate of a small region of chromosome 21. In this case, these genes may not be obligatory effected by mutations in the full length chromosome. However, it is more than likely that the genes are transcribed from within the APP gene, either in certain subsets of cells, or only when the promoters are sporadically activated by toxic intra/inter cellular factors produced in neuronal cells or toxic substances that enter such cells from the external environment. Other mutations in chromosome 21 which affect the configuration of DNA sequences in APP exons 16 and 17 and have been linked to early onset AD (Mullan, M. and Crawford, F. Trends neurosci.16:398-403 1993), may occur also in the alzas genes. The Hardy VI and VG mutation, the VF mutation, and the Dutch EQ mutation that occurs in exon 17 (Selkoe,D.J. A Rev. Neurosci. 17:489-517 (1994); Hardy, J. Clin. Geriatr. Med. 10:239-247 1994)), cause a single amino acid change in ALZASp/p1/p2. However, the DM to QL mutation in exon 16 (Cai, X.D. et al. Science 259:514-516 1993) eliminates the normal orf for ALZASp and ALZAS1p which can lead to translation and expression of alternative proteins using other reading frames. Two hypotethical neuropeptides ("[hyp]ALZASp") SEQ ID:NO 13, shown in figure 1 Hii, and ("[hyp1]ALZASp") SEQ ID:NO 14, shown in figure 1 Hiii, can be translated from alzas cDNA. Amino acid 23 -40 in [hyp1]ALZASp] is identical to the sequence of [hyp]ALZAS2p. If expressed, the hypotethical proteins might play a role in the etiology of AD, and also lead to variations of the disease phenotype e.g., the mutation at codon #713 that causes schizophrenia, Jones, C.T.,et al. Nature Genet. 1: 306-309 (1992)), and the mutation at codon #702 that leads to hereditary cerebral haemorrhage, Dutch type (Levy, E. et al.Science:248

1124-1126 (1990)), may cause alterations in the biochemical property of at least one of the hypotethical proteins.
The following data driven deductions have been made concerniing AD

ALZASp has structural similarities to small pore-forming proteins. Pore-forming proteins found in a variety of organisms penetrate cellular membranes and mediate membrane damage, usually at a very low effector/target cell ratio. For a pore forming protein to be effective, two interconvertible configurations of the protein must exist in the cell: (i) for membrane insertion the protein must present a hydrophobic domain on the surface which must not be dominant, (ii) whereas for traversing the cytoplasm to reach the membrane the protein must behave as a soluble entity. The interconvertible configurations might be achieved in several ways including oligomerization, or interaction through hydrophobic sites with a chaperon. In order to enter the membrane, on contact with the membrane dissociation of the oligomer occurs which re-exposes the protein hydrophobic domain and drives the membrane insertion. ALZASp has the APP transmembrane signal; therefore, like APP, ALZAS can be translocated across the membrane of the endoplasmic reticulum (ER) and the cytoplasm and can insert into the same sites as APP in plasma membranes.

Internally, in response to adherence of certain infectious agents, or secretory products of such agents, to localized areas on cell membranes specific cytoskeleton proteins become highly phosphorylated by activated tyrosine phosphatases and form organised cytoskeleton structures. The latter interacts with the cell membrane directly below the point of contact on the surface membrane and this facilitate entry of the agent into the cytoplasm. Tau may be specifically mobilized and hyperphosphorylated in response to ALZAS interacting with membranes of specific sub sets of neurons. Externally, the entry of ALZAS into neuronal plasma membrane causes the release and binding of IgG, IgM and other immunological factors to the

membrane bound protein, particularly the unique c-terminal sequence. This acts as a signal for the initiation of the complement cascade which targets cells bearing ALZAS for destruction and attempted removal.

In summary, the clinical symptoms of AD are initiated by a combination of ALZASp caused loss of APP from neuronal ER and plasma membranes and slow dissolution of the affected neuronal membranes by complement directed activity against ALZASp on the surface of the membrane. The latter leads to an autoimmune-like condition in those section of the brain affected by the disease.

The clinical symptoms of DS, which are not related to AD, are caused by interaction of DSAS with other certain chromosomal regions which leads to loss or inactivation of genes in the affected regions due to abnormal chromosome condensation.

The Uses of the Molecules of the Present Invention.

A. Diagnostic Uses

ALZASp and DSAS are expressed in all humans clinically positive for AD and DS, respectively. ALZAS is also expressed in 2 out of 5 humans 65 years or older that are clinically normal for AD (some of which have other neurodegenerative diseases) whereas ALZAS has not been found in humans that are distinctly normal, i.e. humans <50 years old without any visible symptoms or history of the same. However, ALZAS has been found in saliva of menopausal women suffering from osteoporosis accompanied by depression. The detection of these molecules may be done by any of a variety of immunological methods (Yolken, R.H., Rev. Infect. Dis. 4:35 (1982); Collins, W.P., In: Alternative Immunoassays, John Wiley & Sons, NY (1985); Ngo, T.T. et al., In: Enzyme Mediated Immunoassay, Plenum Press, NY (1985); incorporated by reference herein.

In one embodiment, the affinity purified monospecific antibodies ALZab1, ALZab2, ALZab3 and ALZab4, based on the sequences in table 1b that we have made can be used in any immuno assay test system to detect DSASp, ALZASp, ALZASp1 and ALZASp2 in dot blot methods or in quantitative methods using sandwich ELISA and trap ELISA techniques as outlined in figures 5 A+B.

In another embodiment the presence of, dsas, alzas, alzas1 and alzas2 mRNA in a cell or in the fluid as is described herein can be determined by any means capable of detecting mRNA encoding these proteins.

Such nucleic acid based assays may use either DNA or RNA to detect dsas, alzas alzas1 and alzas2 mRNA. In one embodiment, the assays may be performed on RNA that has been extracted from blood cells as described in the specifications herein. The assays may be done in situ on biopsied tissue using for example PCR (Mullis, K.B., Cold Spring Harbour Symp. Quant. Biol. 51:263-273 (1986); Saiki, R.K., et al., Bio/Technology 3:1008-1012 (1985); Mullis K. et al., U.S. Patent 4,683,202; Erlich, H., U.S. Patent 4,582,788; Saiki, R. et al., US 4,683,194 and Mullis, K.B., et al., Met. Enzymol. 155:335-350 (1987), transcription-based amplification systems (Kwoh D et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173 (1989); Gingeras TR et al., PCT appl. WO 88/10315; Davey, C. et al. European Patent Application Publication no. 329,822), etc.

B. Prognostic Uses

The present invention additionally provides a capacity to predict very early whether an individual has Alzheimer's disease and if a newly conceived fetus will be born with Down syndrome. Thus, any of the above-described assays may be performed on an asymptomatic individual in order to assess the restaging of these diseases.

C. Therapeutic Uses

Significantly, the present invention provides a means for treating AD and DS. Such treatment may be either "prophylactic" or "therapeutic." A prophylactic treatment is one that is provided in advance of any clinical symptom of AD or DS in order to prevent or attenuate any subsequent onset of the disease. A therapeutic treatment is one that is provided in response to the onset of a symptom of AD or DS, and serves to attenuate an actual symptom of the disease.

In one embodiment, such treatment is provided by administering to a patient in need of such treatment an effective amount of an antibody, or an antibody fragment (F(ab'), F(ab')2, single chain antibodies, etc.) that is capable of binding to, ALZASp (aa 67-79), ALZASp1 (aa 18-51), ALZASp2 (aa 32-44) and DSASp (aa 1-27).

The immunotherapy can be used in the form of an active vaccine, e.g. multiple antigenic epitopes based on the ALZAS sequence, or of a passive vaccine, e.g. humanized antibodies (see humanized antibodies in the following references which are incorporated by reference (Morrison, S.L., Science, 229:1202-1207 (1985); Oi, V.T. et al., BioTechniques 4:214 (1986); Jones, Ü.T. et al., Nature 321:552-525 (1986); Verhoeyan et al., Science 239:1534 (1988).

In another embodiment the desired therapy may be obtained by targeting the nucleic acid molecules, specifically the promoter sequences Pdas 1-6 and Palzas 1-14 of the present invention with "antisense" nucleic acid molecules. Antisense oligonucleotides are disclosed in European Patent Application Publication Nos. 263,740; 335,451; and 329,882, and in PCT Publication No. WO90/00624, all of which references are incorporated herein by reference. Used herein, an aniti-sense nucleotide is a DNA or RNA whose sequence is complementary to the sequence of PDS 1 to PDS 6 or PALZ 1 to PALZ 14 described herein, such that it is capable of binding to, or hybridizing with, an endogenous promoter or heat shock sequence in a manner sufficient to impair its transcription, and significantly inactivate it in a cell; or

whose sequence is complementary to alzas- nucleotide 201-240, or alzas1- nucleotide 55-156, or alzas2- nucleotide 96-135 or dsas- nucleotide 88-104, and thereby impair (i.e. attenuate or prevent) its the translation into protein. These molecules may be transported into the cell using the Protein-Polycation Conjujates system (Beug, H. et al United States patent 5,354, 844 11/10 1994) in an appropriate pharmaceutical compound, also see Oldham, R.K. (In: Principles of Biotherapy, Raven Press, NY, 1987), and Ledley, F.D., In: Biotechnology, A Comprehensive Treatise, volume 7B, Gene Technology, VCH Publishers, Inc. NY, pp 399-458 (1989)); all of which references are incorporated herein by reference. The principles of the present invention can be used to provide a prophylactic gene therapy to individuals who, due to inherited genetic mutations, or somatic cell mutation, are predisposed to Alzheimer's disease or down syndrome.

Thus, in one embodiment of this invention, an antisense oligonucleotide that is designed to specifically block transcription or translation of a dsas, alzas, alzas1 or alzas2, mRNA transcript can be used to impair the expression of DSASP, ALZASp, ALZAS1p or ALZAS2p in a cell, and thereby provide a treatment for DS or AD.

In yet another embodiment such treatment is provided by administering to the patient an effective amount of a cys-cys reducing reagent which will inhibit or reduce the oligomerization of ALZASp or ALZASp2.

III. Administration of the Molecules of the Present Invention

Additional pharmaceutical methods may be employed to control the duration of action of any of the aforementioned reagents used to treat AD and DS. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

PUZZLING QUESTIONS ANSWERED BY THIS INVENTION

The discovery that the genes which encode Aß might be separate

from APP mRNA has provided insight into how, and why, mutations at different regions of the APP gene, including regions outside the Aß encoding region, cause AD. It has also provided an explanation for the AD and DS like symptoms produced in transgenic animals expressing APP mRNA, and in mice in which APP mRNA transcription was blocked.

As indicated in Figure 2D a TATA/cap correlated promoter system and a "CCAAT" box located in exon 15 (figure 1 O) can program the expression of a longer isoform of ALZAS which terminates at the usual APP mRNA poly A site. This transcript expresses a protein which we call truncated n-terminal APP" (Tn-APP). Tn-APP contains Aß, APP TM-II and APP C-terminal. APP TM-II can cause the symptoms of AD by out competing APP for membrane anchor sites. Also indicated in figure 2d is the location of an orf "pac" on the complementary strand of APP mRNA. The nucleotide sequence of pac is shown in figure 1 E, SEQ ID:NO 9. Pac can be transcribed from two correlated promoter systems, "P1PAC" and "P2PAC" SEQ ID:NO 8 located 5' upstream of the orf (figure I D). pac mRNA encodes a hypotethical 103 amino acid protein "PAC" figure 1 F, SEQ ID:NO 10. PAC aa 1-23 are identical to DASP aa 1-23. The latter sequence can be released by a constitutive cleavage site located between PAC amino acids 23 and 24. The truncated PAC sequence can mimic many of the biochemical activities of DSAS. mRNAs encoding pac and Tn-APP was shown to be transcribed from APP mRNA cloned into HL 60 cells by probing northern blots with a nucleotide sequence that overlapped the 21 nucleotides encoding the first 8 amino acids of both PAC and TN-APP (data not shown).

SEQ ID:NO 9

5' ATGTCGGAATTCTGCATCCATCTTCACTTCAGAGCTCTCCTCCGTCTTGAT
ATTTGTCAACCCAGAACCTGGTCGAGTGGTCAGTCCTCGGTCGGCAGCAGG
GCGGGCATCAACAGGCTCAACTTCGTTTTCTGTGTTGGCTGGCACAGAGTC
AGCCCCAAAAGAATGCCACGGCTGGAGATCGTCCAGGCTGAACTCTCCATT
CACGGGAAGGAGCTCCACGGTGGTTTTCGTTTCGGTCAAAGATGGCATGAG
AGCATCGTTTCCGTAACTGATCCTTGGTTCACTAATCAAGTTGGCCAAGAC
GTCATCTGA 3'

SEQ ID:NO 10

M S E F C I H L H F R D L L R L D I C Q W P R T W S
S G Q S S V G S R A G I N R L N F V F C V G W H R V
S P K R M P R L E I V Q A E L S I H G K E L H G G F

R F G Q R W H E S I V S V T D D W F T N H V G Q D V I

This invention has permitted us to construct a comphrensive model of AD.

THE MODEL.

Internally, in response to adherence of certain infectious agents or secretory products of such agents to localized areas on cell membranes, specific cytoskeleton proteins become highly phosphorylated by activated tyrosine phosphatases and form organised cytoskeleton structures. The latter interacts with the cell membrane directly below the point of contact on the surface membrane and this facilitate entry of the agent into the cytoplasm. Tau may be specifically mobilized and hyperphosphorylated in response to ALZAS interacting with membranes of specific sub sets of neurons. Externally, the entry of ALZAS into neuronal plasma membrane causes the release and binding of IgG, IgM and other immunological factors to the membrane bound protein, particularly the unique c-terminal sequence. This acts as a signal for the initiation of the complement cascade which targets cells bearing ALZAS for destruction and attempted removal.

Based on our results, the above considerations and results reported by others in the literature, we have proposed the following model for AD which shows how the various AD related proteins interact to produce the AD phenotype. The model can be rigorously tested.

STAGING OF AD

1. ALZAS transcription and expression is activated by any of a number of external and intracellular factors including stress, viral and other pathogenic invasions and environmental toxins, in a specific cell type (perhaps lymphocytes). ALZAS molecules aggregate to remain soluble, traverse the cytoplasm using the same route as APP and are secreted.

2. Secreted ALZAS, because of the unique c-terminal which is expressed from intron sequences is treated as foreign (i.e. an endogenous pathogen). Soluble antibody IgM, IgG, IgA (anti-ALZAS Ig), produced in response to ALZAS bind to and neutralize ALZAS.

3. Conditions that decrease the efficiency of the immune system e.g. certain viral infections, physiological aging etc, permit an increasing number of ALZAS molecules to escape destruction. These molecules interact with cell membranes of specific sub populations of neurons (target cells) and enter and damage the membrane.

4. Interaction of ALZAS with target neuron membranes triggers two major physiological responses. (i) Activation of tyrosine phosphatases which convert tau to PHF-tau. The latter interacts with the cytoplasmic side of the membrane below the points of interaction with ALZAS and form long neurofibrillary structures which facilitate entry of ALZAS into the cytoplasm and (ii), Activation of the complement system which attempts to destroy ALZAS. Because the specific amino acid sequences targeted by complement protease are identical in ALZAS and APP, APP is also cut by the ALZAS directed protease/s. The complement destruction of neuronal membrane APP results in a local acute inflammatory reaction and autoimmune-type inflammatory destruction of the affected neurons.

5. ALZAS interacts with ApoE proteins in a way that hides the hydrophobic domain and allows the complex to remain soluble in the cytoplasm; in this manner ALZAS is chaperoned (transported in a soluble form) between the neuronal cytoplasm the ER and the neuronal plasma membrane. If ALZAS oligomerize with ApoE2, and to a lesser extent ApoE3, Cys-Cys interaction occurs between the two molecules which tends to maintain ALZAS in a soluble form in which the protein cannot traverse the ER.

6. If ALZAS oligomerize with ApoE4 which does not have a Cys residue, on contact with the ER membranes ALZAS regains the

monomeric state in which the hydrophobic membrane signal is exposed and the protein competes with, replaces and prevents APP from occupying sites on the ER. normally ER bound APP remains in the cytoplasm where it is degraded by cellular proteolytic activity into smaller fragments including various Aß species.

7. PS1/PS2 form part of a highly specific APP binding site in neuronal ER membranes. Mutations in any part of the PS1/2 molecule which have the slightest effect on conformation or mutations in APP which occur close to the transmembrane signal or within it, considerably diminishes the specificity of interaction between APP and the PS1/PS2 component of the binding site and confers an even higher preference for ALZAS binding to the APP transmembrane site. Whereas APP is translocated and carries out all its essential biochemical activities, ALZAS probably causes dissolution of the membrane which results in loss of function of a number of ER membrane proteins.

8. Aß which is perhaps the most protease resistant portion of the APP protein, and elongated, sticky, neurofibrils produced from PHF-tau which form tangles, are released into intracellular spaces as the neuronal membranes "dissolve".

Having now generally described the invention, through references and examples that makes it more readily understood by any one sufficiently skilled in the art, it must be pointed out that these are not intended to be limiting of the present invention, unless specified.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential

features herein before set forth and as follows in the scope of the claims.

At present using ALZab2 in the immunoblot procedure described earlier, we are detecting ALZASp in blood samples taken from one out of five (5 of 19) people who were undergoing cognitive and other test given to people having signs that are suspected to be associated with the onset of AD. The detection was done on 1μl whole blood taken from specimens which were freshly drawn from the patients for other routine determinations associated with the institution's AD test routine. Using the same procedure as above, the antibody detects ALZASp in 100% of the blood specimens taken from AD victims very soon after death.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bergmann, Johanna &
Preddie, Rick

(ii) TITLE OF INVENTION: AGENTS FOR PRESYMPTOMATIC DIAGNOSIS AND THERAPEUTIC TARGETING IN HUMAN ALZHEIMER'S DISEASE AND DOWN SYNDROME

(iii) NUMBER OF SEQUENCES:

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DR. J. BERGMANN
(B) STREET: MÖRIKESTR. 22
(C) CITY: HAMBURG
(D) STATE:
(E) COUNTRY: GERMANY
(F) ZIP: 22587

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #125

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: NONE
(B) REGISTRATION NUMBER:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (4940) 862-576
(B) TELEFAX: (4940) 862-596

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: dsas
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGTCGGAAT TCTGCATCCA TCTTCACTTC AGAGATCTCC TCCGTCTTGA 50

TATTTGTCAA CCCAGAACCT GTATTACATC ATAA 84

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: dsas
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAAATAATAA TAAAAGGCTT TAAAAAGAGC ATTAATTACC TGGTAGAACA 50

AAACTGCTAC TTTGGGGCTT TGTT 74

INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: protein
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: DSAS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Glu Phe Cys Ile His Leu His Phe Arg Asp Leu Leu Arg Leu
1 5 10 15

Asp Ile Cys Gln Pro Arg Thr Cys Ile Thr Ser
20 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PDS1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGGATTTTAT ATAACTACTA GAG 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE: PDS2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTTAAGC CACATAAAGT GTTCTGTAAA ACCAAGCAGT CTATG 45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PDS3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCACTTTAAG TCCGCACTCA T 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PDS4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTGAAAGAAC TATACATACA GCCTCTTTCT TTTCT 35

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PDS5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TAAATCATAA AACGGGTTTG TTTCTTCCCA CATTATTCTA TAAATTGCTG 50

TGGCGGGGG 59

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PAC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGTCGGAAT TCTGCATCCA TCTTCACTTC AGAGCTCTCC TCCGTCTTGA 50
TATTTGTCAA CCCAGAACCT GGTCGAGTGG TCAGTCCTCG GTCGGCAGCA 100
GGGCGGGCAT CAACAGGCTC AACTTCGTTT TCTGTGTTGG CTGGCACAGA 150
GTCAGCCCCA AAAGAATGCC ACGGCTGGAG ATCGTCCAGG CTGAACTCTC 200
CATTCACGGG AAGGAGCTCC ACGGTGGTTT TCGTTTCGGT CAAAGATGGC 250
ATGAGAGCAT CGTTTCCGTA ACTGATCCTT GGTTCACTAA TCAAGTTGGC 300

CAAGACGTCA TCTGA

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PAC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Glu Phe Cys Ile His Leu His Phe Arg Asp Leu Leu Arg Leu
1 5 10 15

Asp Ile Cys Gln Pro Arg Thr Trp Ser Ser Gly Gln Ser Ser Val Gly
20 25 30

Ser Arg Ala Gly Ile Asn Arg Leu Asn Phe Val Phe Cys Val Gly Trp
35 40 45

His Arg Val Ser Pro Lys Arg Met Pro Arg Leu Glu Ile Val Gln Ala
50 55 60

Glu Leu Ser Ile His Gly Lys Glu Leu His Gly Gly Phe Arg Phe Gly
65 70 75 80

Gln Arg Trp His Glu Ser Ile Val Ser Val Thr Asp Asp Trp Phe Thr
85 90 95

Asn His Val Gly Gln Asp Val Ile
100

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:alzas

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATGGATGCAG AATTCCGACA TGACTCAGGA TATGAAGTTC ATCATTCAAA     50
AATTGGTGTT CTTTGCAGAA GATGTGGGTT CAAACAAAGG TGCAATCATT    100
GGACTCATGG TGGGCGGTGT TGTCATAGCG ACAGTGATCG TCATCACCTT    150
GGTGATGCTG AAGAAGAAAC AGTACACATC CATTCATCAT GGTGTGGTGG    200
AGGTAGGTAA ACTTGACTGC ATGTTTCCAA GTGGGAATTA A             241
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: ALZASp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

```
Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu
                5                  10

Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp
        15                  20

Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met
25                  30                  35

Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile
            40                  45

Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser
    50                  55                  60

Ile His HIs Gly Val Val Glu Val Gly Lys Leu Asp
                65                  70

Cys Met Phe Pro Ser Gly Asn
```

75

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:ALZASp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Gln Asn Ser Asp Met Thr Gln Asp Met Lys Phe Ile Ile
5 10

Lys Asp Trp Cys Ser Leu Glu Lys Met Trp Val Gln Thr Lys
15 20 25

Val Gln Ser Leu Asp Ser Trp Trp Ala Val Leu Ser
30 35 40

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: Yes

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: ALZASp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gln Asn Ser Asp Met Thr Gln Asp Met Lys
5 10

Phe Ile Ile Lys Asn Trp Tyr Val Lys
15 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 156 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:alzas1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
ATGGATGCAG AATTCCGACA TGACTCAGGA TATGAAGTTC ATCATCAAAA   50
ATTGGTACGT AAAATAATTT ACCTCTTTCC ACTACTGTTT GTCTTGCCAA  100
ATGACCTATT AACTCTGGTT CATCCTGTGC TAGAAATCAA ATTAAGGAAA  150
AGATAA                                                  156
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: ALZAS1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Met Asp Ala GLu Phe Arg His Asp Ser Gly Tyr Glu Val
                5                   10

His His Gln Lys Leu Val Arg Lys Ile Ile Tyr Leu Phe
    15                  20                  25

Pro Leu Leu Phe Val Leu Pro Asn Asp Leu Leu Thr Leu
```

30 35

Val His Pro Val Leu Glu Ile Lys Leu Arg Lys Arg
40 45 50

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTGATAATTA AATGTTATAG CATGGACACT GACATTTACA TTTTTTACTT 50
ATGTTTTTGG TTTTTAAATG ACTCTGCAT 79

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTATTATTT GAATAATGAA ATTCATCAGA ACAATTA 37

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single
(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:PALZ3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCAATTTATA GAAAAGGAAG AGTTCGTAGG TTATAAATTC TGTTAGTTGC 60
TAAGAAGCAT TTTTAAAA 68

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single
(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:PALZ4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATGCTCATTT TTAAAGGCTT TTATTATTAT TTCTGAAGTA ATGAGTGCAC 50
ATGGAAAAA 59

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid

(D) TOPOLOGY: linear
(C) STRANDNESS: single
(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:PALZ5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TATTCCAGGA ACAAATCCTT GCCAACCTCT CAACCAGG 38

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single
(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:PALZ6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TAGCATGTAT TTAAATGCAG CAGAAG 26

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(C) STRANDNESS: single
(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:PALZ7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GAAGGTTTAA ATATAGGGTA TCATTTTTCT TTAAGAGTCA TTTATCAATT 50
TTCTTC 56

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE:alzas2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATGTGGGTTC AAACAAAGGT GCAATCATTG GACTCATGGT GGGCGGTGTT 50
GTCATAGCGA CAGTGATCGT CATCACCTTG GTGATGCTGA AGAAGAAACA 100
GTACACATCC ATTCATCATG GTGTGGTGGA GGTAGGTAAA CTTGACTGCA 150
TGTTTCCAAG TGGGAATTAA 170

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: ALZAS4

Met Val Gly GLy Val Val Ile Ala Thr Val Ile
5 10

Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln
15 20

Tyr Thr Ser Ile His His Gly Val Val Glu Val
25 30

Gly Lys Leu Asp Ser Trp Trp Ala Val Leu Ser
35 40

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: ALZAS5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Val Trp Gln Thr Lys Val Gln Ser Leu Asp Ser Trp
5 10
Trp Ala Val Leu Ser
15

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE:PALZ9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CCAAATAAAG AGCAAGAATA AAGCAACATT TCA 33

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
CLONE: PALZ10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTATGCTTTA AAAAGCAATA CA 22

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TCCTTTCTTT CAGAATGCCT ATTCCTGTGC ATTAAAAGTG TCCCTCC 47

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTTAAAGTAA GCATCAAA 18

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTTTTTATAT AACCTCATCC AAATGTCCCC TGCATTTAA 39

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(B) CLONE: PALZ14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAAAATGAAA TTCTTCTAAT TGCGTTTATA AATTGTAATT A 41

Figure 4

A = description of antigen trap ELISA test
B = description of antibody trap ELISA test
C-E Detection of expression of DSAS and ALZAS related proteins ALZASp (ALZAS), ALZASp1 (ALZAS1) and DSASp (DSAS) in 2μl of human blood dotted on nylon membranes using chemiluminescent detection on system. For dot blots, proteins extracted from brain and lymphocyte by ammonium sulphate precipitation were dialysed and boiled for 5 minutes in 5% SDS. 2μl of the SDS treated proteins were spotted onto nylon membranes, treated with the appropriate antibody and detected with chemiluminescent system:
(C) dots were probed with antibody ALZab1; dots 1= normal lymphocytes, 2= DS brain, 3= DS lymphocytes, 4 & 5 = trisomy 21 fibroblast, 6 = normal brain, 7 = AD cortex, 8 & 9 = AD lymphocytes, 10 = normal fibroblast;
(D) 1.5 μl serum obtained from ten normal women over 35 years old selected at random was probed with ALZab1.
(E) detection of endogenous anti-DSAS IgG in serum and saliva of DS patients using the antibody trap ELISA test well 1 = positive control; well 2 & 3 = saliva from DS patients; wells 4 & % serum from DS patients; well 6 = negative control.
(F) Dot blots of serum from 28 autopsy confirmed AD victims was probed with ALZab2 (at least 50% of these patients we incorrectly diagnosed for a condition other than AD before death);D 6 & 7 = positive control; D 9 & 10 = negative control
(G) Dot blots of serum from suspected early AD patients, patients with depression and autopsy confirmed AD victims: A 1= physiological aging; B 1 = Parkinsons disease, A 2-4, 6-7, B 3, 5, C 2, 3 = AD with depression; A 5, 8, B 2 ,3, 5, 7, 8, 9, 10 = suspected AD, C 1, 6-10 = serum from confirmed AD; D 1-10 serum from normal humans.
(H) Detection of endogenous anti-ALZAS IgG in patients blood, serum and saliva: (i) serum samples from Japanese patients who died in hospital in 1995,filled circles = serum from AD patients; open circles = autopsy confirmed humans without AD; (ii) serum from patients diagnosed as AD and clinically normal people selected at random: filled circles = AD patients; empty circles = clinically normal persons; (iii) serum from people with M > L related FAD, open circles = family members without the mutation without clinical symptoms AD; filled circles = family members with the mutation with no clinical signs of AD; large circle with filled interior family member with the mutation and with clinical AD.
(I) western blots; proteins in ammonium sulphate precipitates were electrophoresed on 17.5 % acrylamide gels, blotted onto nylon filters, reacted with the appropriate antibody and detected with the immuno-chemiluminescent system, 1. proteins were isolated from DS lymphocyte and treated with ALZab1; 2. proteins were isolated from AD hippocampus and treated with antibody ALZab2; 3. proteins were isolated from AD hippocampus and treated with ALZab3.

Figure 5A-D
(A). Isolation of ALZAS from cortex obtained from a single

Figure 4 cont'd.

autopsy confirmed AD patient. The protein was affinity purified on anti ALZab2 antibody linked to CNBR-sepharose. The gel was blotted onto nylon a nylon membrane and the protein detected with a chemiluminescent detection system.
(B) Silver stain of SDS gel: proteins obtained following affinity purification of ALZAS from serum from a late stage AD patient. In addition to ALZAS, ALZAS bound to various immunoglobulins and to APOE was detected on the silver stained gel and confirmed by specific staining duplicate gels
(C) Detection of ALZAS-IgG complexes present on gel of 5B with anti-human IgG (Fc fragment).
(D) Western blot of cationic non SDS polyacrylamide gel following electrophoresis of serum from (a) a patient with sporadic AD, and (b) a patient with swedish mutation AD. The gels were reacted first with anti ALZASab1 and then with the chemiluminescence system. ALZAS was detected in a and ALZAS2 in b. The upper band in a is ALZAS and in b is ALZAS2 both complexed to human IgG. ALZAS and ALZAS2 have slightly different isoelectric points which can be seen on the gels.

TABLE 1a

PCR primers used to detect mRNA in post mortem brains and lymphocytes.

Primers:

ds254	product size expected = 254bp
Forward	TTCATATGGTGAGTCATGTCGG
Reverse	GTAATTGAAGTTTTAAATATAG
alz287	product size expected = 287 bp
Forward	GTGGACAAATATCAACACCGAGGAG
Reverse	ACATAGTCTTAATTCCCACTTGG
alz393	product size expected = 393 bp
Forward	GTCCTGCATACTTTAATTATGATG
Reverse	AGCCATCATGGAAGCACACTGATTCG
alz188	product size expected = 188bp
Forward	GTGGACAAATATCAAGACGGAGGAG
Reverse	TCCTTAATTTGATTTCTAGCACAGG
alz267	product sise ectected = 267 bp
Forward	TCCTGCATACCTTTAATTATGATG
Reverse	TTCATGGTAATCCTATAGGCAAC
alz148	product size expected = 148 bp
Forward	GTGTTCTTTGCAGAAGATGTGGG
Reverse	ACATAGTCTTAATTCCCACTTGG

Table 1b

Antibodies prepared against the following epitopes were used to detect proteins from post mortem brains and lymphocytes.

* = Cys residue added to facilitate conjugation KLH.

ALZab1	LHFRDLLRDLIC*
ALZab2	VGKLDCMFPSGNC*
ALZab5	EVGKLDC
ALZab3	RKIIYLFPLLFVLP*
APZab4	KVQSLDSWWAVLSC*

WHAT IS CLAIMED IS:

1. A nucleic acid molecule, "dsas", substantially free of natural contaminants, that encodes a protein "DSASp".

2. The nucleic acid molecule of claim 1 that encodes "DSASp" wherein said nucleic acid molecule has the sequence SEQ ID NO:1.

3. A protein, substantially free of natural contaminants, chosen from the group "DSASp" and "PAC" wherein said protein has the sequence SEQ ID:NO 3 and SEQ ID:NO 10 respectively.

4. A nucleic acid sequence capable of specifically transcribing the nucleic acid molecule of claim 1 wherein said nucleic acid "PDS1", "PDS2", "PDS3" and "PDS4" are promoters or regulatory elements and have the sequence, SEQ ID:NO 4, SEQ ID:NO 5, SEQ ID:NO 6 or SEQ ID:NO 7, respectively.

5. A nucleic acid sequence "PADS" which functions as the poly A addition signal required for termination of transcription of the nucleic acid molecule of claim 2, wherein said nucleic sequence has the sequence SEQ ID:NO 2.

6. A reagent or combination of reagents capable of detecting specifically the presence of the nucleic acid molecule of claim 2 in human blood, amniotic fluid, or other human tissue fluids wherein said nucleic acid has the sequence SEQ ID:NO 1,

7. A reagent or combination of reagents capable of specifically detecting the presence of a protein of claim 3, in human blood, amniotic fluid, urine, saliva, brain or spinal fluid wherein said protein has the sequence SEQ ID:NO 3 and SEQ ID:NO 10.

8. A method for diagnosis of Down syndrome in humans and especially in pregnant women, according to claim 6, wherein the reagent is a nucleic acid based molecule.

9. A method for diagnosis of Down syndrome in humans and especially in pregnant women, according to claim 7, wherein the reagent is an antibody or an anti-ALZASp peptide molecule.

10. A method for treating Down syndrome in humans which is based on any of the following: (1) prevention or modulation of the transcription of alzas by inhibition of the activity of PDS1,

PDS2, PDS3, or PDS4 the nucleic acids of claim 4, or PDAS, the nucleic acid of claim 5, either by use of blocking molecules or by deletion or substitution of nucleotides within said nucleic acid sequences; (2) prevention of translation of DSASp from dsas, the nucleic acid molecule of claim 2, either by use of blocking molecules or by deletion or substitution of nucleotides within said nucleic acid molecule; and (3) inhibition of the activity of DSASp by use of a polyclonal, monoclonal or humanized antibody prepared against DSASp or Pac.

11. A nucleic acid molecule "alzas", substantially free of contaminants, which encodes a protein selected from the group, "ALZASp", "ALZASp1", "ALZASp2".

12. A nucleic acid molecule "alzas1", substantially free of contaminants, which encodes a protein "ALZASp1".

13. The nucleic acid molecules of claim 11 (specifically nucleotide sequence number 251 - 3'end, and claim 12 (specifically nucleotide sequence number 54- 3'end) having the sequence SEQ ID:NO 11 and SEQ ID:NO 15 respectively, that encode proteins ALZASp, ALZASp2, [hyp1]ALZASp, [hyp2]ALZASp, [hyp1]ALZASp2 having the sequence, SEQ ID:NO 12, SEQ ID:NO 13, SEQ ID:NO 14; SEQ ID:NO 25 and SEQ ID:NO 26 respectively.

14. A fragment of a nucleic acid molecule which contains a sequence capable of specifically transcribing the nucleic acid molecules: of claim 11, alzas, and of claim 12, alzas1, wherein such nucleic acid fragments, "PALZ1", "PALZ2", "PALZ3", "PALZ4", "PALZ5", "PALZ6", "PALZ7" and "PALZ8" are promoter elements or other transcriptional regulator elements having the sequence: SEQ ID:NO 17, SEQ ID;NO 18 SEQ ID:NO 19, SEQ ID:NO 20, SEQ ID:NO 21, SEQ ID:NO 22, SEQ ID:NO 23 and SEQ ID:NO 24.

15. The fragments of claim 14 capable of transcribing the nucleic acid molecule "alzas2" having the sequence SEQ ID:NO 25, and [hyp1]ALZASp2 having the sequence SEQ ID:NO 26, wherein said nucleic acid fragments "PALZ9", "PALZ10", "PALZ11", "PALZ12", "PALZ13" and "PALZ14" are promoter elements and other transcriptional regulators having the sequence, SEQ ID:NO 27, SEQ ID:NO 28, SEQ ID:NO 29, SEQ ID:NO 30, SEQ ID:NO 31 and SEQ ID:NO 32.

16. A reagent capable of detecting the presence of a molecule selected from the group consisting of alzas, alzas1, ALZASp ALZASp1, ALZASp2, ALZASp3, ALZASp4, ALZASp5 in humans.

17. The reagent of claim 16, wherein said reagent is a nucleic acid molecule capable of detecting the mRNA (particularly

ONE OF EIGHT

FIGURE 1 A

5′
ATGTCGGAATTCTGCATCCATCTTCACTTCAGAGATCTCCTCCGTCTTG
*
ATATTTGTCAACCCAGAACCTGTATTACATCATAA./..391bp../
GAAATAATAATAAAAGGCTTTAAAAAAGAGCATTAATTACCTGGTAGAAC
AAAACTGCTACTTTGGGGCTTTGTT
3′

FIGURE 1 B

M S E F C I H L H F R D L L R L D I C Q P R T C
I T S

FIGURE 1 C

5′
CCACGATTTGATTTATCTCCATTTCTAAGCTTTTGGTAAAGGATTTTATA
TAACTACTAGAGCTGACCACAAAGGAGATGTGGCTTTTTGATTTACTCCA
ATTCTAACATTAGGGGGGAAAAGTGATTTCATCTGAAAAGGAAGCTGCTG
TCTTCTTTGCACAAACCTTTTGATGAATAACACTATCGGGAACATCATGG
GGTCCTGCTCACTCCATGATCTTACTCTTGCAAGAGAAGTTAAATCATGG
GAGGTAGGGGAGGGAATGAAAAGAAAGAGCTGGGGGAGAAATGAATGGGT
AAAGGAAGACAGTTCCGGATGTGAATTTAAGCCACATAAAGTGTTCTGTA
AAACCAAGCAGTCTATGCATGTGTTTGATCCTTCAAATAAACCTATTCTA
AACCTATTTTCCCAGAAAACACAGTGGAAGTCAAAGTGGCTGCTATATTG
AAATTAATTCCACTTTAAGTCCCGACTCATGCACCAACTTTGCTGCCTTG
TAGTCTGAAAGAACTATACATACAGCCTCTTTCTTTTCTTTTTTTTTTTT
GGGATGGAGTCTCGCTCTGTCGCCTCGGCACGATCTCAGTCTACTGCAAC
3′

FIGURE 1 D

5′
TGTAGTATAGAGACCAAAATGTAAAGAGAGATAGAATACATTACTGATGT

GTGGATTAATTCAAGTTCAGGCATCTACTTGTGTTACAGCACAGCTGTCA

AAAGGCGATAATGAGTAAATCATAAAACGGGTTTGTTTCTTCCCACATTA

TTCTATAAATTGCTGTGGCGGGGGTCTAGTTCTGCATCTGCTGAAAGAAC

TTGTAGGTTGGATTTTCGTAGCCGTTCTGCTGCATCTTGGACAGGTGGCG

CTCCTCTGGGGTGACAGCGGCGTCAACCTCCACCACACCATGATGAATGG

ATGTGTACTCTTTCTTCTTCAGCATCACCAAGGTGATGACGATCACTGTC

GCTATGACAACACCGCCCACCATGAGTCCTATGATTGCACCTTTGTTTGA

ACCCACATCTTCTGCAAAGAACACCAATTTTTGATGATGAACTTCATATC

CTGAGTC
3′

FIGURE 1 E

5′
ATGTCGGAATTCTGCATCCATCTTCACTTCAGAGCTCTCCTCCGTCTTGAT

ATTTGTCAACCCAGAACCTGGTCGAGTGGTCAGTCCTCGGTCGGCAGCAGG

GCGGGCATCAACAGGCTCAACTTCGTTTTCTGTGTTGGCTGGCACAGAGTC

AGCCCCAAAAGAATGCCACGGCTGGAGATCGTCCAGGCTGAACTCTCCATT

CACGGGAAGGAGCTCCACGGTGGTTTTCGTTTCGGTCAAAGATGGCATGAG

AGCATCGTTTCCGTAACTGATCCTTGGTTCACTAATCAAGTTGGCCAAGAC

*
GTCATCTGA
3′

FIGURE 1 F

M S E F C I H L H F R D L L R L D I C Q P R T W S S

G Q S S V G S R A G I N R L N F V F C V G W H R V S

P K R M P R L E I V Q A E L S I H G K E L H G G F R

F G Q R W H E S I V S V T D D W F T N H V G Q D V I

3/25

THREE OF EIGHT

FIGURE 1 G

5'
AGGTTCTGGGTGGACAAATATCAAGACGGAGGAGATCTCTGAAGTGAAGA

TGGATGCAGAATTCCGACATGACTCAGGATATGAAGTTCATCATCAAAAA

TTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGTGCAATCATTGG

ACTCATGGTGGGCGGTGTTGTCATAGCGACAGTGATCGTCATCACCTTGG

TGATGCTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAG

GTAGGTAAACTTGACTGCATGTTTCCAAGTGGGAATTAAGACTATGAGAG

AATTAGGCTTAGCTTTTTGCTAAGAACTAGCTAAGTATCTCTTTTAAAAA

ACCAATCAGTGTGCTTCCATGATGCTTGGGTTACAGTTGTTCTTTCTTGT

TTTGGTTTTCATTCATTGCAACTTACCGTGAATATTCTGCTCAAGGTATT

GAGAGTGTGTGTTGTTATCTTAACTTACAATTTGTGTTGAAGTTATCAAA

TAATACAAATGATAATGCATGACTTTAAAAAAGCAT
3'

FIGURE 1 H

(i)
M D A E F R H D S G Y E V H H Q K L V F F A E D V G

S N K G A I I G L M V G G V V I A T V I V I T L V M

L K K K Q Y T S I H H G V V E V G K L D C M F P S G

N

(ii)
M Q N S D M T Q D M K F I I K N W Y V K

(iii)
M Q N S D M T Q D M K F I I K N W C S L Q K M W V

Q T K V Q S L D S W W A V L S

4/25

FOUR OF EIGHT

FIGURE 1 I

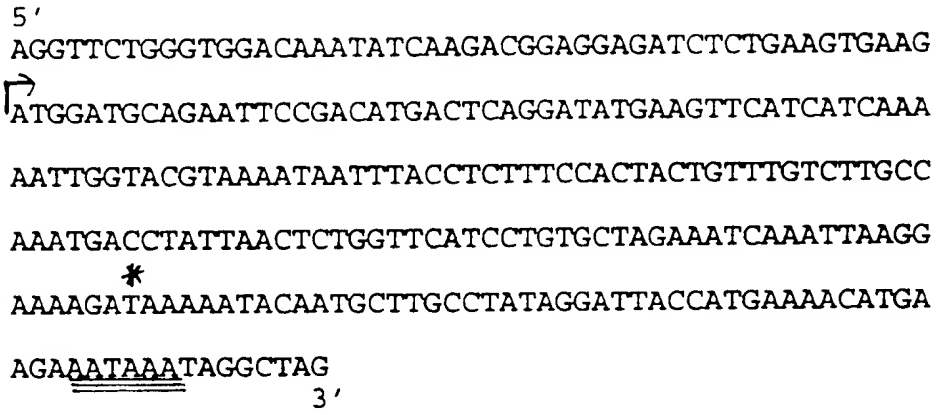
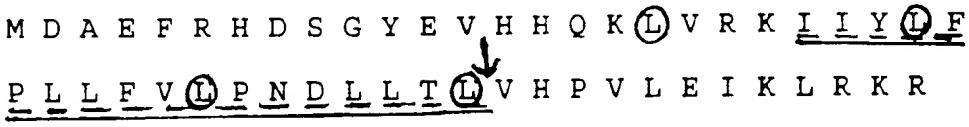


FIGURE 1 J



5/25

FIVE OF EIGHT

FIGURE 1 K

5'

TTGATAATTAAATGTTATAGCATGGACACTGACATTTACATTTTTTACTT

ATGTTTTTGGTTTTTAAATGACTCTGCATTTTGTTTTAAGCTTCAAATTA

TTATTTGAATAATGAAATTCATCAGAACAATTAGTGTTAAGAATCATATA

GCAATTTATAGAAAAGGAAGAGTTCGTAGGTTATAAATTCTGTTAGTTGC

TAAGAAGCATTTTTAAAATTATGTACTATAGCTCTTTATTCAGCAGACGA

ACCAATTACAATCTGTGTAACTAGAACACTTGATCAAAATTATATAATTT

TTACAACGCTTCACTGCATAGATACATGAACATAATTTATTTTGAATTGG

AACAAAGCCCCAAAGTAGCAGTTTTGTTCTACCAGGTAATTAATGCTCAT

TTTTAAAGGCTTTTATTATTATTTCTGAAGTAATGAGTGCACATGGAAAA

AGACACATAATAGGCTAAACAATAAGCCCGTAAGCCAAGCCAACATATTC

CAGGAACAAATCCTTGCCAACCTCTCAACCAGGATTTAACTTCTGCTTTT

CCCCCATTTTCAAAAATTATAGCATGTATTTAAATGCAGCAGAAGCCTTA

CTTTCAGGTTTCCCTTACCCTTTCATTTCTTTTTGTTCAAAATAGGTAGT

AATTGAAGGTTTAAATATAGGGTATCATTTTTCTTTAAGAGTCATTTATC

AATTTTCTTCTAACTTCAGGCCTAGAAAGAAGTTTTGGGTAGGCTTTGTC

TTACAGTGTTATTATTTATGAGTAAAACTAATTGGTTGTCCTGCATACTT

TAATTATGATGTAATAC

6/25

SIX OF EIGHT

FIGURE 1 L

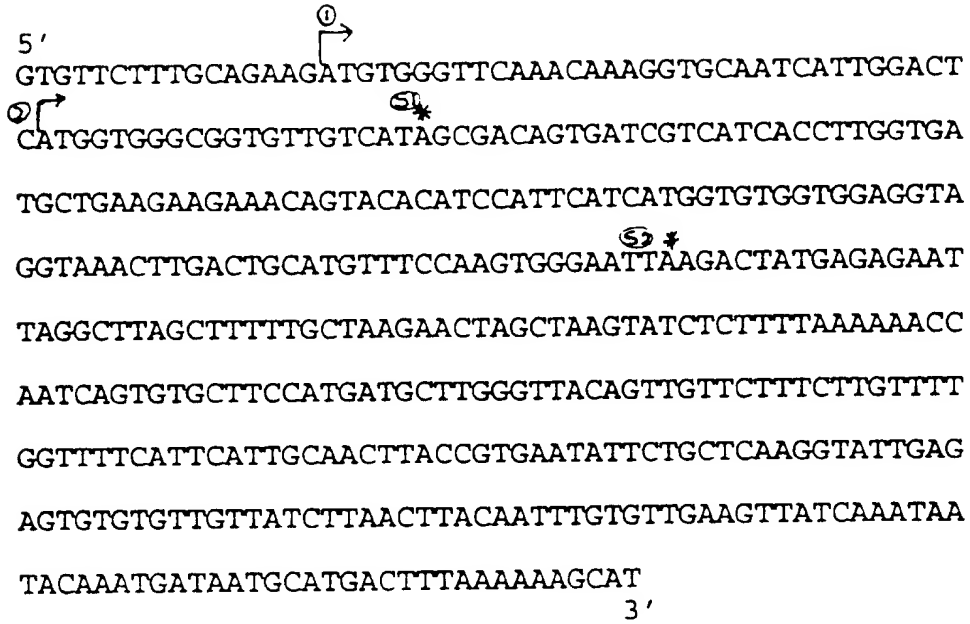
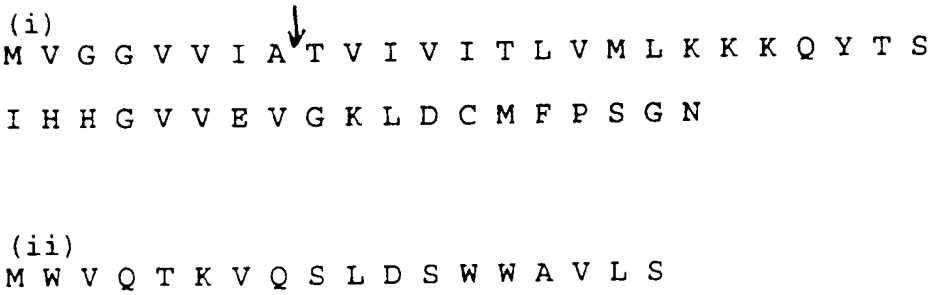


FIGURE 1 M



7/25

SEVEN OF EIGHT

FIGURE 1 N

5'
TATTTTTATGTTTAATCCAAATAAAGAGCAAGAATAAAGCAACATTTCAGAT
TTTGGTTTCTGGAGACAATAGTTAGAAAGCATGAGTTATGATTGACTTAAAA
TTCTTGTTGCCTGTACTTCACTTTGAAATAACATTATGCTTTAAAAAGCAAT
ACACTGCTAAAGGTTAATTTGAATTCTGCAGAATTACTATAGCAAAAAGTAG
GTAACAAGATATCTTTTTTTCTATTGTTTAACTCCTTTCTTTCAGAATGCCT
ATTCCTGTGCATTAAAAGTGTCCCTCCAAGGAAATTAGGACATCTGCAGAGT
TGAAAAACACCTAAGTCTCAGTCACTTAGAGTCACACATCAGGGCTCAGAGT
GCTATGACTAGGAAAATGCTGACCTCCTTTCATTAGTATGATCGTTCCTTTC
CAGCTTTTGATAGATCCAAGCGCTATCTTCCCACCACTCACCAAATGTTCCA
CCTGTCAAAGGGTTTCAGGTCCCTGCAGACTTCGGTTTTGACCTGTGGGGAA
AGTAGACTTCCTCGAACTGGGGAAGCCACATGTTGTACATCCTTCTATAAAC
TATGATTATCATTCTTAGTAGGAAAATATGTGATTTCTTTTTTTTTTTTTTT
TTTTAAAGTAAGCATCAAATATTTGACCAACCAGTTGGGCAGAGAATATACT
GAAACTTTTTATATAACCTCATCCAAATGTCCCCTGCATTTAAGAAATGAAA
TTCTTCTAATTGCGTTTATAAATTGTAAATTATATTGCATTTAGAAATTAAA
ATTCTTTTTCTTAATTTGTTTTCAAGG
3'

EIGHT OF EIGHT 9/25

FIGURE 1 O

5'
GTTATGACACACCTCCGTGTGATTTATGAGCGCATGAATCAGTCTCTCT
CCCTGCTCTACAACGTGCCTGCAGTGGCCGAGGAGATTCAGGATGAAGT
TGTGAGCTGCTTCAGAAAGAGCAAAACTATTCAGATGACGTCTTGGCCA
ACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCCTATGCC
ATCTTTGACCGAAACGAAAACCACCGTGGAGCTCCTTCCCGTGAATGGA
GAGTTCAGCCTGGACGATCTCCAGCCGTCCGATTCTTTTGGGGCTGACT
CTGTGCCAGCCAACACAGAAAACGAAGTGAGCCTGTTGATGCCCGCCCT
GCTGCCGACCGAGGACTGACCACTCGACCAGGTTCTGGGTTGACAAATA
TCAAGACGGAGGAGATCTCTGAAGTGAAGATG
3'

Fig 2 a

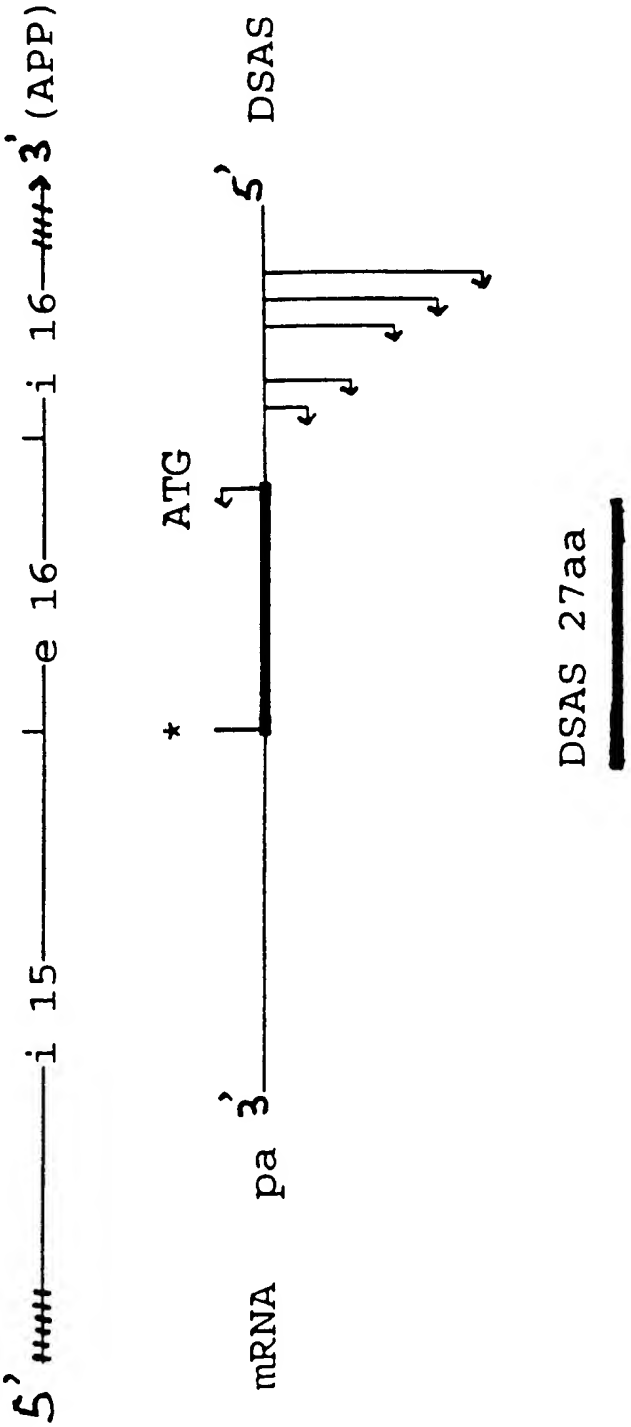


Fig 2 c

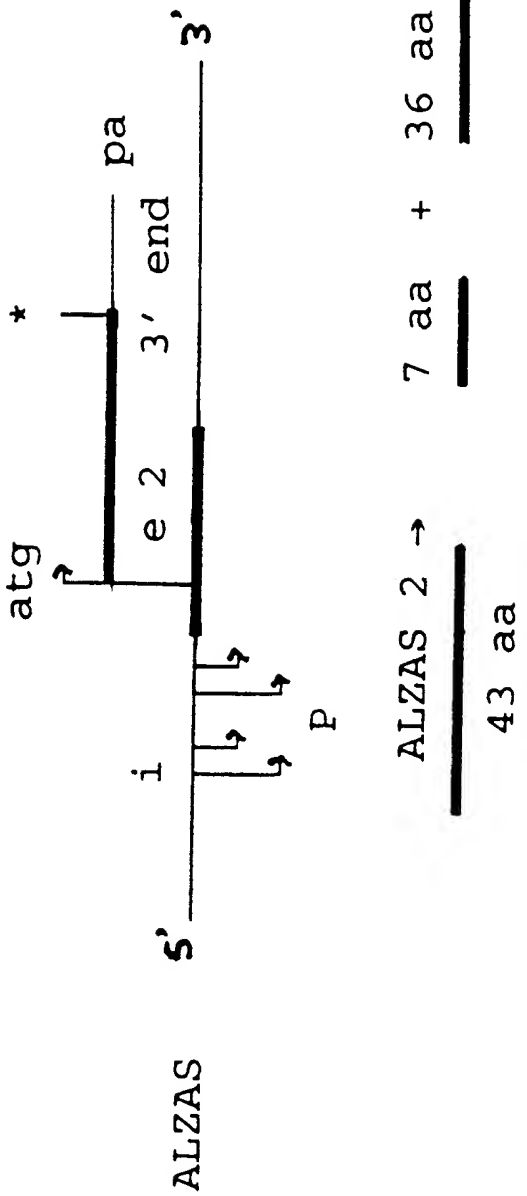


Fig. 2 d

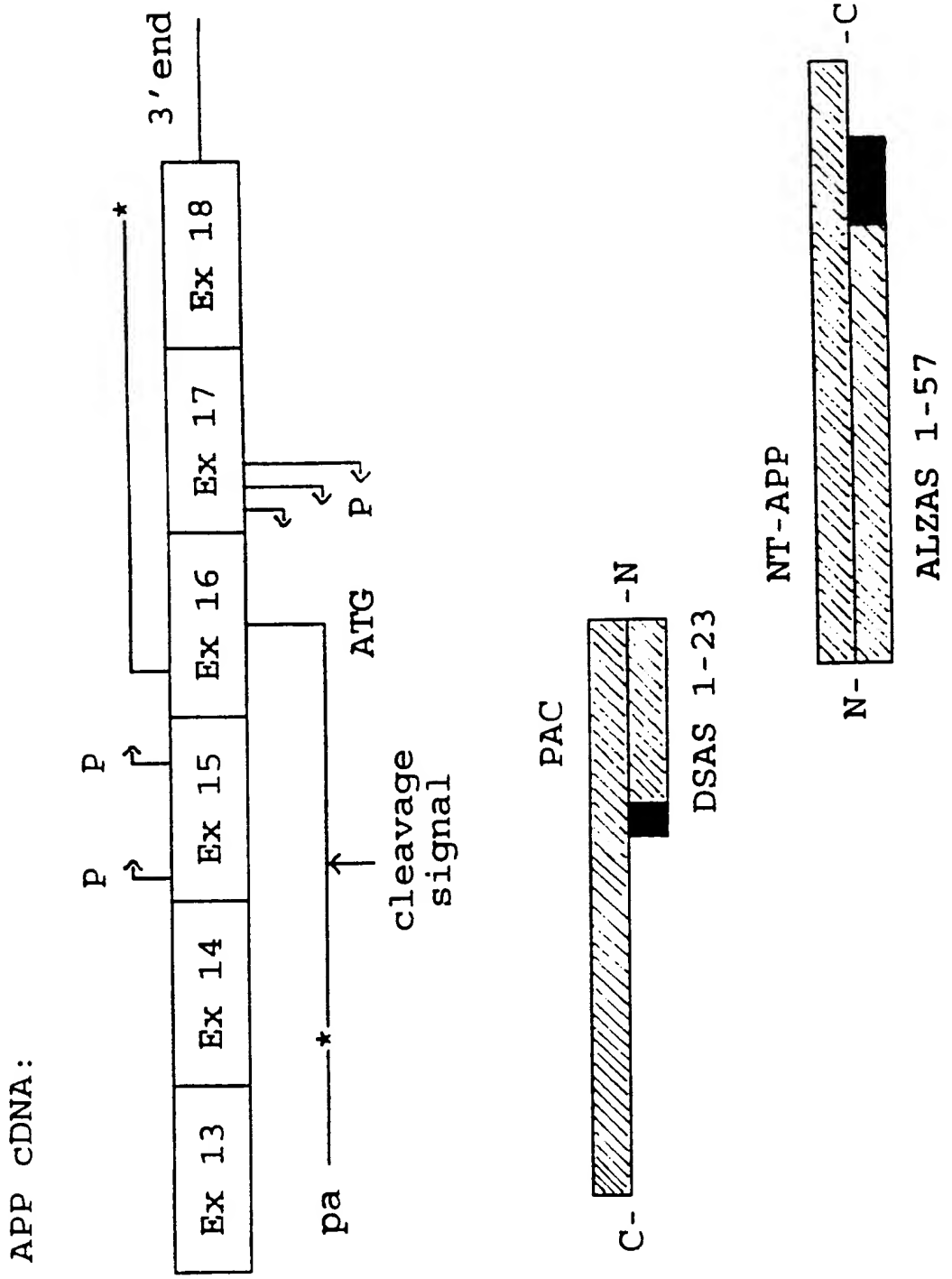

In APP cDNA:
Ex 13
Ex 14
Ex 15
Ex 16
Ex 17
Ex 18
3'end
P
P
P
ATG
cleavage signal
pa
PAC
-N
C-
DSAS 1-23
NT-APP
-C
N-
ALZAS 1-57

Figure 3

A

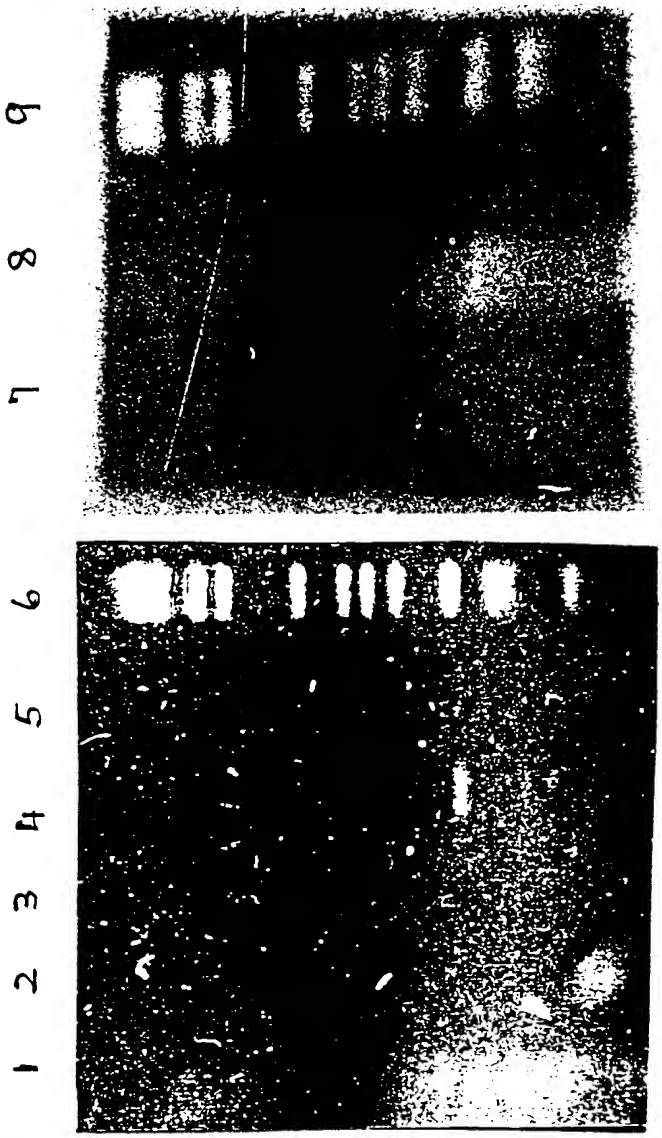
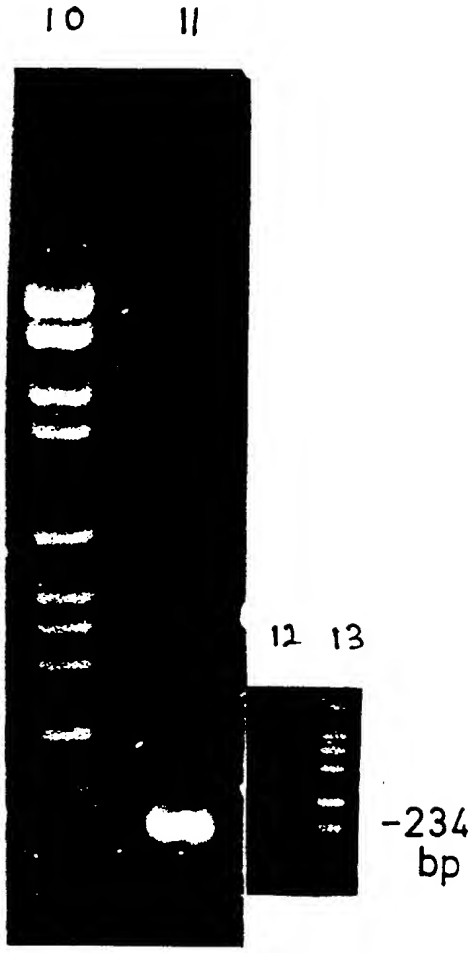


Figure 3

B.



C.

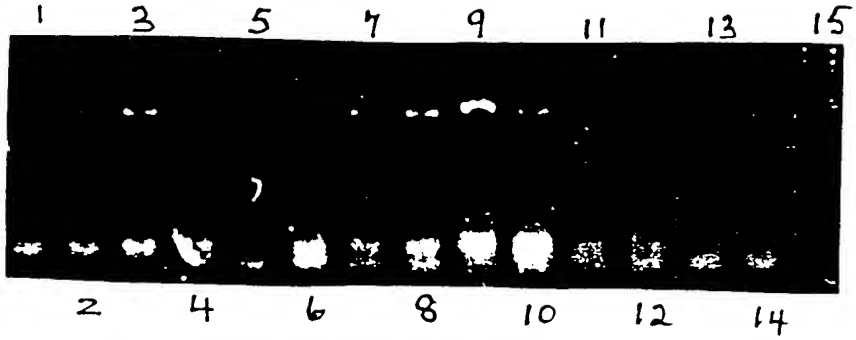
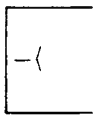
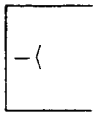


Figure # 4 16/25

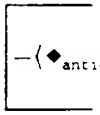
ELISA-test for ALZAS protein



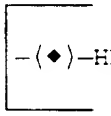
(0) Coat plates with anti-ALZASa



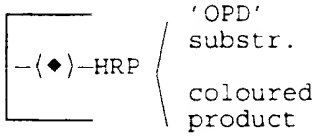
(1) block with 1% non-fat dry milk.



(2) React with antigen (ALZAS) in serum, urine, saliva, protein extracts etc.



(3) React with anti-ALZASb IgG conjugated with horse radish peroxidase



(4) React with substrate ortho-phosphate diamine or other suitable substrate; substrate is cleaved to give a colour reaction which is proportional to the amount of bound HRP-conjugated (second) antibody; measure concentration of colour in microplate reader.

Fig 2 a (i)

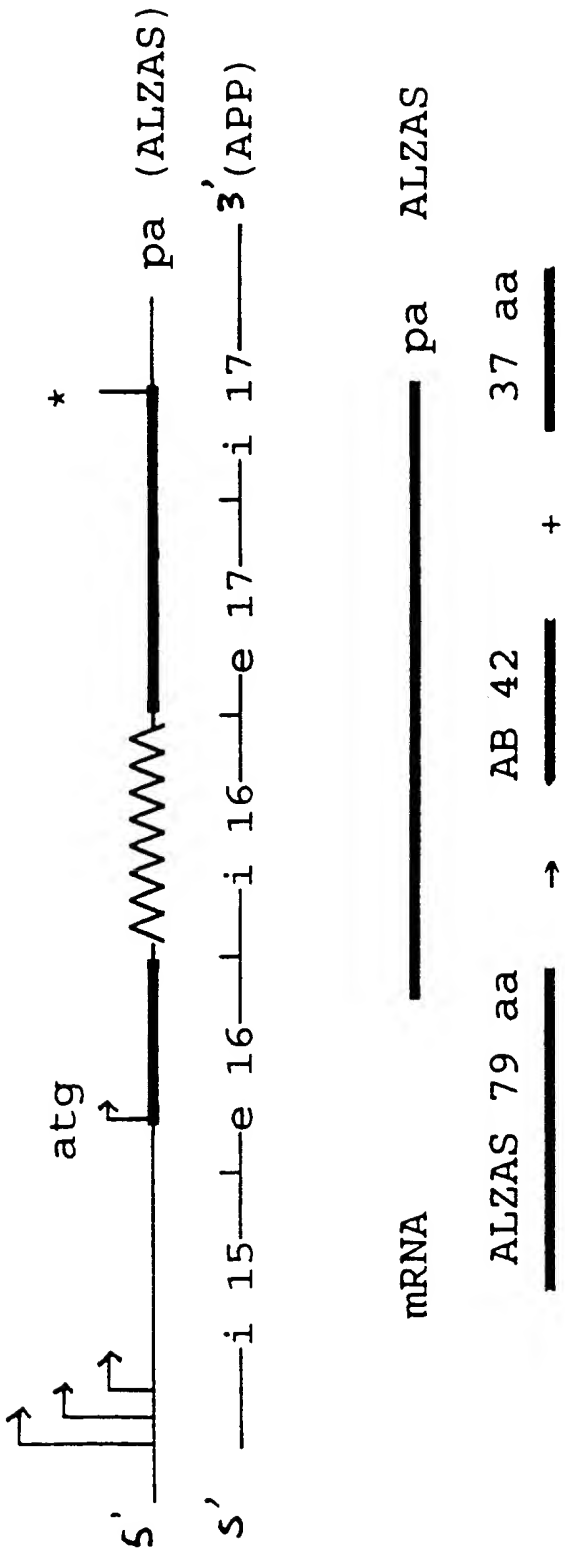
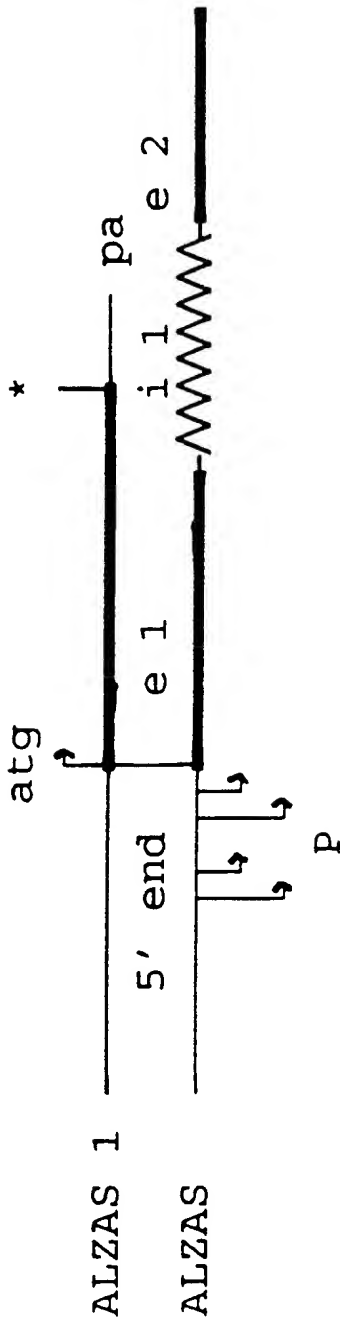

5'
atg
*
pa (ALZAS)
5'
i 15
e 16
i 16
e 17
i 17
3'(APP)
mRNA
pa ALZAS
ALZAS 79 aa
AB 42
37 aa
+

Fig 2 b



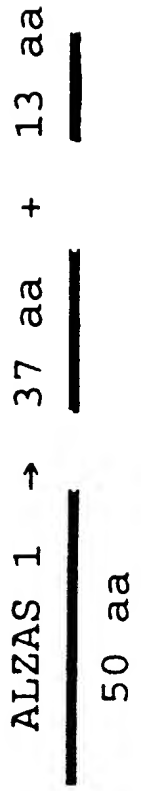


Figure 4

ELISA-test for anti-ALZAS endogenous IgG

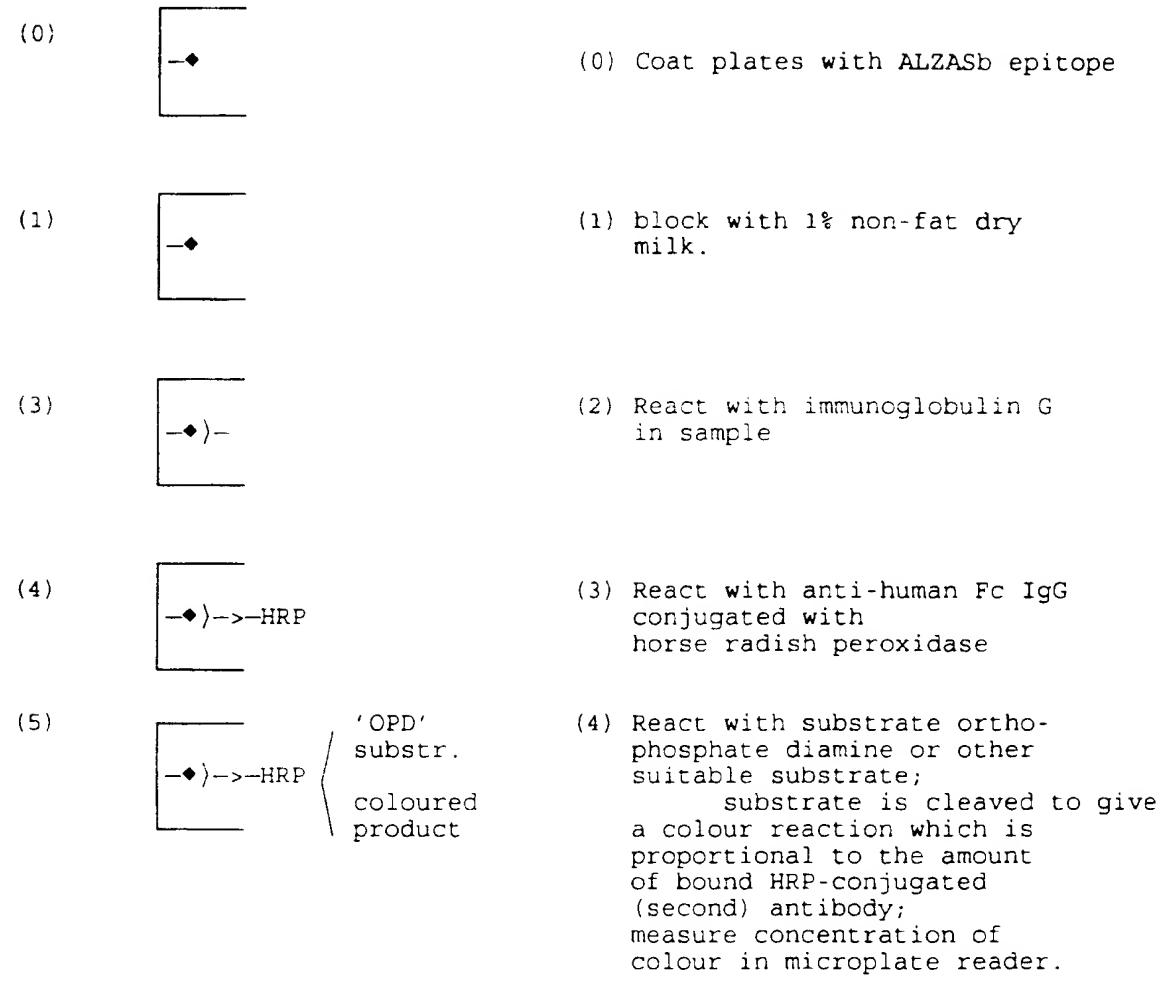
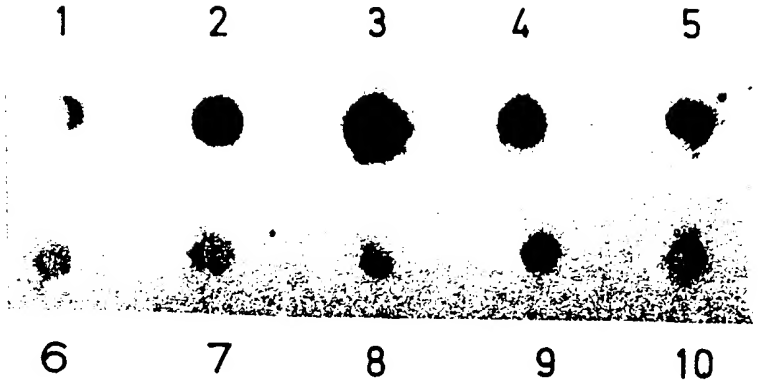
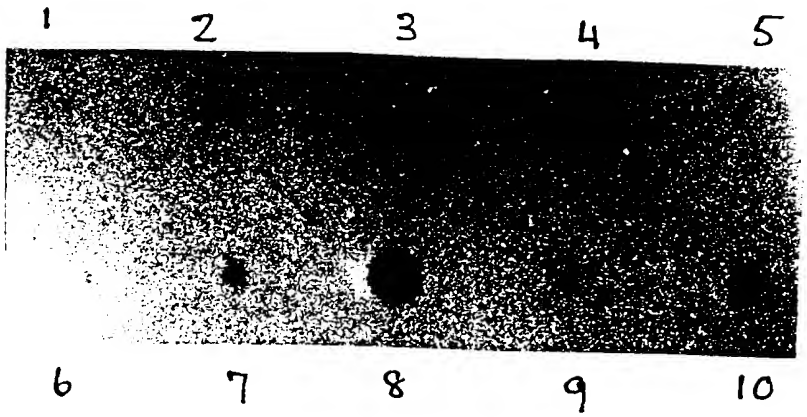


Figure 4

C.



D.



E.

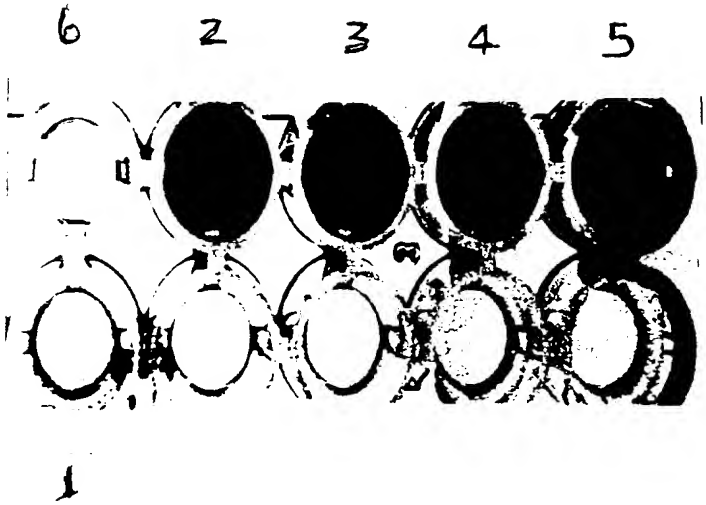


Figure 4

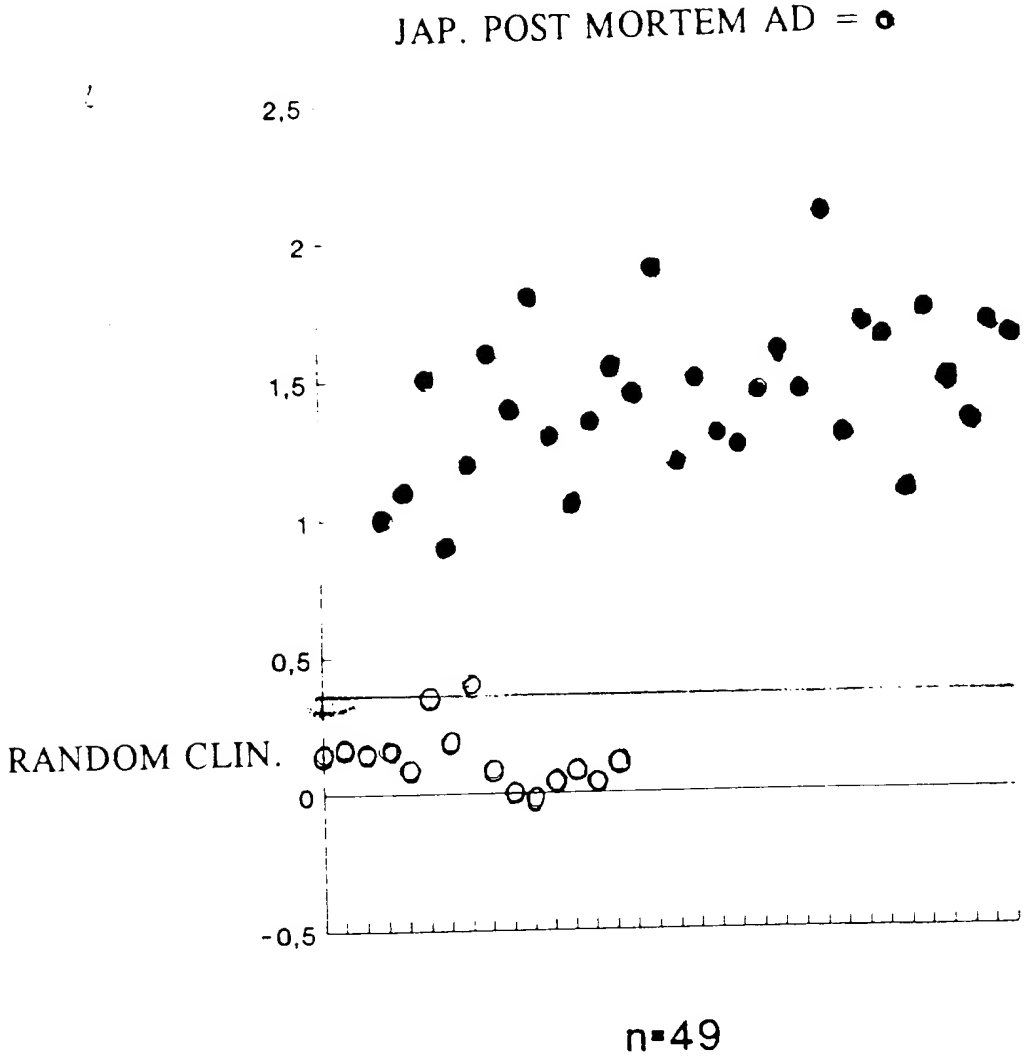

HUMAN SERUM
JAP. POST MORTEM AD = o
2,5
2
1,5
1
0,5
RANDOM CLIN.
0
-0,5
n=49

Figure 4

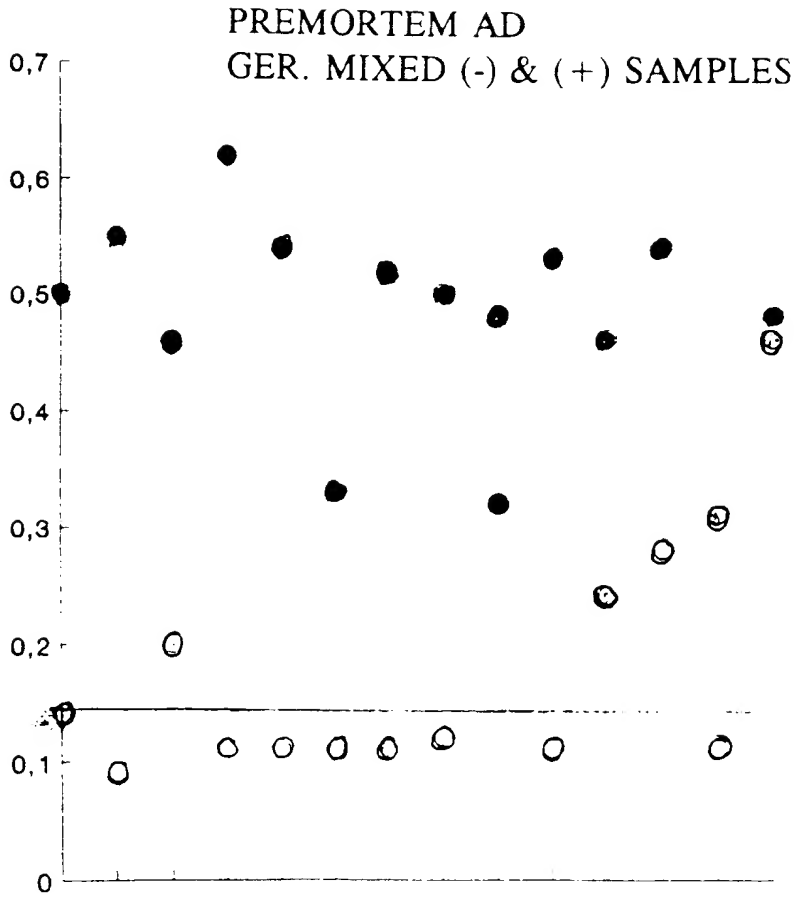
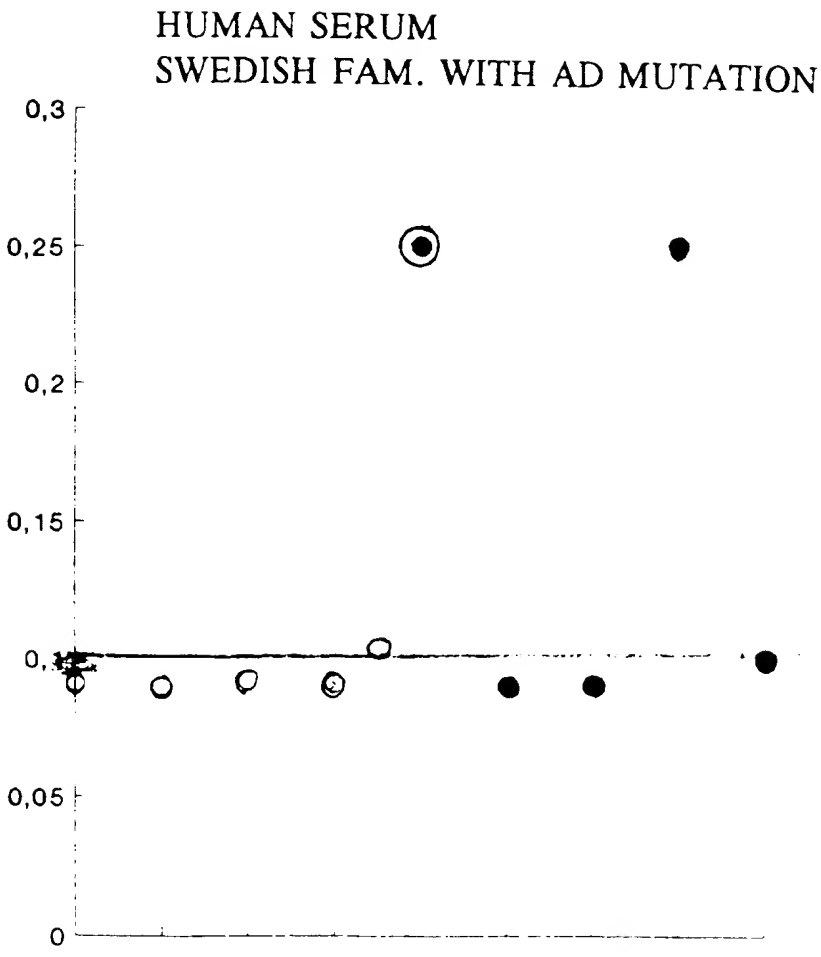


Figure 2



o = CLIN (-) n=10

Figure 4

I.

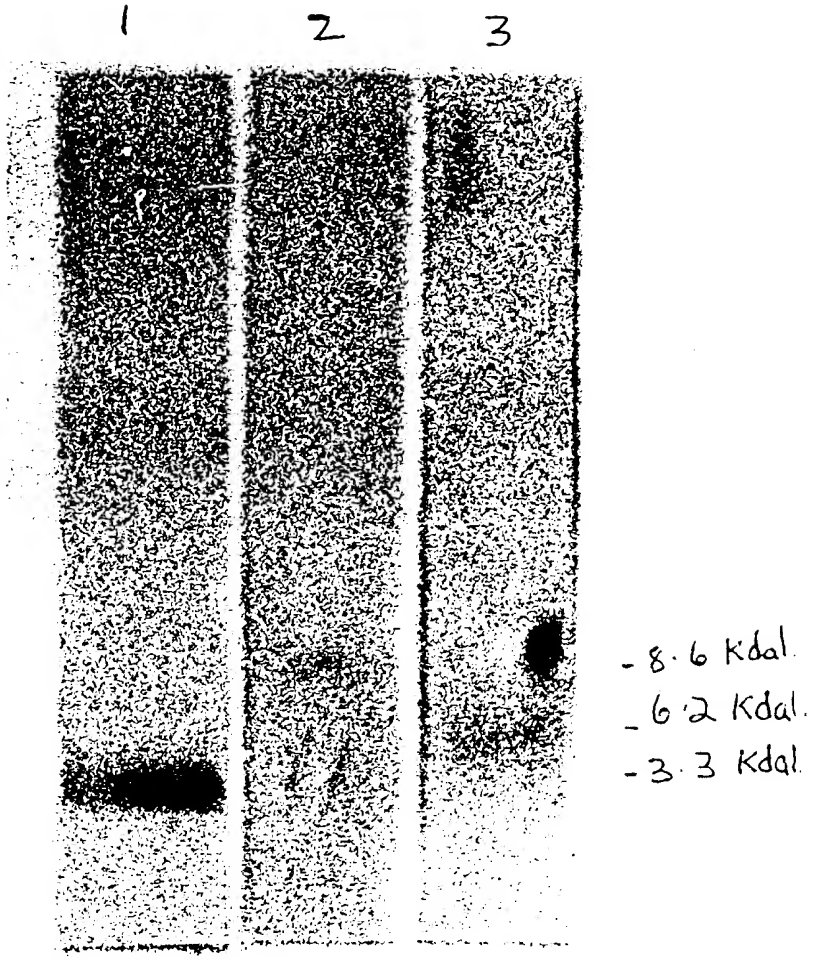


Figure 5

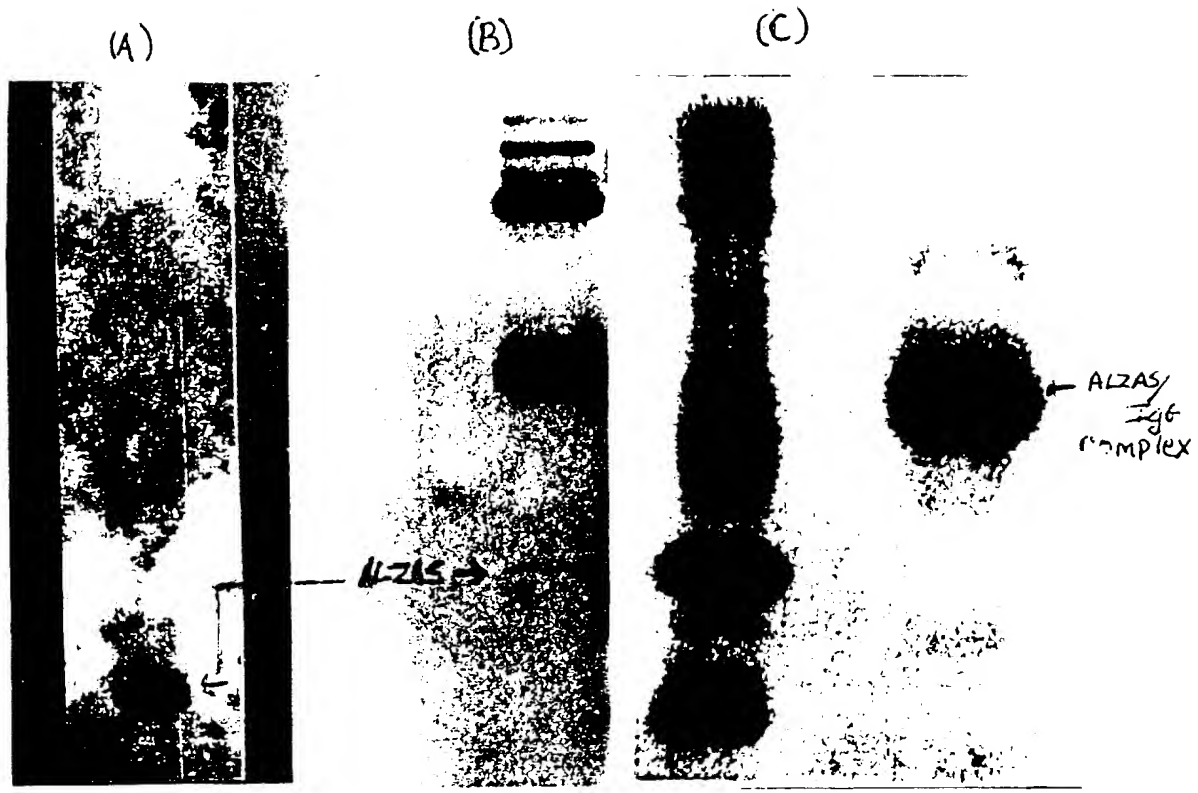


Figure 5

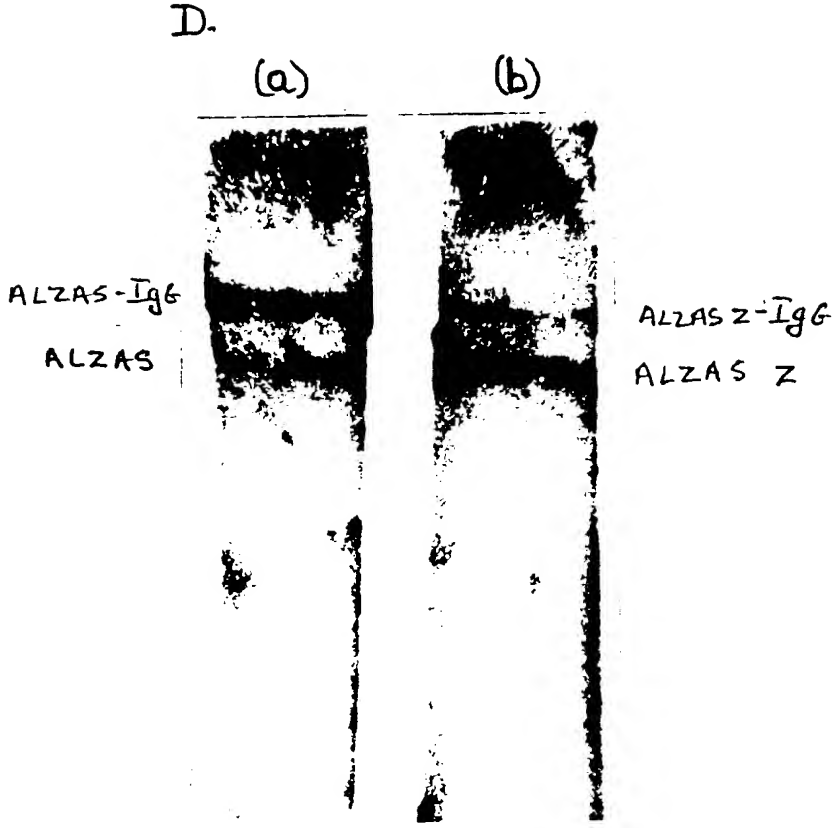


FIGURE 6

(a)

Alignment of the amino acid sequence in the trans membrane helix (TM) of ALZASp with amino acids in the region of TM-2 in S-182 protein and amino acids in the second TM region of STM2.

```
S182              133  SILNAAI  MISVIVVMTILLVVLY  K
                        *   * *  *:  :*: *:::: *   *
ALZASp [APP-TM]    28  SNKGAIIGLMVGGVVIATVIVITLVMLK
                        *    :*  *:  :*: *:::: *   *
STM2              138  SVLNILI  MISVIVVMTVFLVVLY  K
```

bold & italics = point mutation found in this position in S182, STM2 and APP
[APP-TM] = sequence identical to APP transmembrane helix

(b)

Alignment of amino acid sequence in c-terminal end of ApoE/2/3/4 and c-terminal end of ALZASp

```
ApoE4       305  VGTSAAPVPSDNH
                 **      ** *
ALZASp       68  VGKLDCMFPSGN
```

[As = 3.61 (runs=100)]

Alignment of amino acids in the ApoE/2/3/4 LDL receptor binding site/heparin binding site and c-terminal of ALZASp1

```
ApoE        158 LRKLRKRLLR
                  *****
ALZASp1      45 EIKLRKR
```

* = identical amino acid
: = equivalent amino acid